CLINICAL URINARY ANALYSIS

A CRITICAL STUDY

BY

J. C. B. STATHAM

Captain Royal Army Medical Corps

[Reprinted from the "R.A.M.C. Journals" of September, 1903, and February and March, 1904]

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Owing to the considerable period which separated their appearance and unavoidable interruptions to work, the three papers, which are reprinted here, overlap considerably and are obviously incomplete. These papers represent the work of nearly two years, and many attempts to select or originate such processes for quantitative urinary analysis as would be rapid and simple, and accurate enough for clinical purposes. To render the results of the analysis more tangible and appreciable, standards of comparison have been adopted and all analytical results illustrated by means of charts.

The standard of comparison adopted for work in cases of acute disease is that of the ratios found to exist in normal urine between its various constituents—it appears to afford a satisfactory working basis in such cases. In chronic disease —in addition to the use of the standard just mentioned—the total cyclical output of urinary constituents is sometimes compared with the theoretical output on the same diet in health, calculated on the bases of average urinary excretion per kilogramme of body weight. This standard, though open to many objections and of limited applicability, has, I think, some value.

Though very conscious of the defects of these papers I have consented to their reproduction, in the hope that they may be of some use, however small, to those interested in clinical urinary analysis.

J. C. B. Statham.

CLINICAL URINARY ANALYSIS:

A CRITICAL STUDY.

PAPER I.

(*Reprinted from the "R.A.M.C. Journal" of September*, 1903.)

THE importance of the subject supplies me with the best reason I can urge for introducing this somewhat technical paper into the Journal.

The urine, in whose nitrogenous constituents are contained nine-tenths of the nitrogen excreted by the body, and in which the final chapter of the story of nitrogenous body metabolism may be read, does not appear to have received that attention in England which its importance demands. How often one hears the expression used "the urine was normal" or "the urine showed nothing," when only a simple qualitative test for sugar or albumen in the urine had been made. How hasty and misleading such conclusions may sometimes be is evident when we remember that both sugar and albumen may be absent and yet the urine be gravely abnormal, reflecting perhaps profound disturbance of the body metabolism.

A normal urine is one which not only contains no abnormal products, but one in which the normal constituents are proportionately represented, and excreted in quantities in some degree proportional to individual body weight. It is because such is the case that quantitative urinary analysis has value in clinical work, and a knowledge of the quality of the urine becomes an asset in diagnosis and prognosis neither negligible nor unimportant.

So long as our conceptions of quantitative urinary analysis were limited to either the long and laborious processes of the laboratory, when an endeavour was made to obtain extreme accuracy for research purposes, or to the simple but generally inaccurate or misleading "clinical methods," the difficulty of making a urinary analysis practical and of real utility was very great. Experience and research, however, have suggested accurate yet rapid processes, and we appear to have reached a point where quantitative urinary analysis may become a practical help in the diagnosis and prognosis of disease.

As illustrative of the unsuitability of the pure laboratory method and the inaccurate so-called clinical method of quantitative estimation I will give two examples, one of each, taking urea as the urinary constituent estimated.

A clinical method of urea estimation one often sees employed is as follows : 2 or 5 cc. from a sample of urine (generally morning urine) are mixed with some hypobromite of soda solution in a small Doremus ureometer, graduated in percentage of urea, the nitrogen evolved in the process being read off in these percentages. The sources of error in such a process are many : (1) If the urine has been taken from an isolated micturition, its urea percentage gives us no idea of the urea percentage in the cyclical urine, for the richness of the urine in urea may be three of four times as great after a meal, or when the urine is concentrated, as it is in the morning urine ; (2) the hypobromite solution, if not carefully and freshly prepared, may not evolve all the nitrogen of the urea, or may itself evolve oxygen and so vitiate results : the hypobromite solutions used in routine clinical work are not always above reproach ; (3) the nitrogen of other bodies besides urea is liberated by the hypobromite solution, approximately one-third of that of the uric acid and kreatinine, and all the nitrogen of the ammonia present. The hypobromite solution also only liberates 92 per cent. of the nitrogen of urea, unless sugar be present. These two sources of error are not allowed for, because one is supposed to balance the other. This rule is approximately true for normal urine, but quite untrue and misleading when applied to some abnormal urines, in which the nitrogen of the extractives and ammonia may rise to 20 or even 30 per cent. of the total nitrogen, as in diabetes ; (4) no allowance is made for the influence of temperature or pressure on the volume of the gas ; the effect of the former is sometimes considerable. This then is the so-called clinical method of estimating urea—a method so inaccurate as to be not only useless but misleading.

Perhaps the best known and one of the most accurate of laboratory research methods of estimating urea is that of Moerner and Sjöcqvist ; but this process takes all one night and half of the next morning to complete, and consequently could only be used when time was no object. I hope to show, later on, that it is possible to estimate the urea in a reasonable time and, too, with a reasonable degree of accuracy.

I have used the expression " laboratory process " when speak-

ing of Moerner's method of urea estimation, in the sense that it is essentially a method used in research work only; for all quantitative processes must necessarily be laboratory ones though the laboratory be but a modest affair.

For general convenience this paper on urinary analysis has been divided into two parts. The first part is devoted to a description of the schemata employed, with notes upon the methods of analysis adopted: while in the second part, which I hope will appear in a future number of the Journal, it is proposed to consider some work which has been carried out by myself and others in France on similar lines and by methods analogous to the ones described.

On plate I. is shown a draft form of an analysis report, which I have prepared, and which is based on analogous forms in current use in France. In this form of report are two schemata or graphics; some explanation of these graphics will be found on the form itself, but in order to elucidate matters some further explanation of them may be desirable. Such a form as this might be used with advantage in obscure or interesting cases, especially when nutritional disturbance has been a marked feature, particularly as it could be attached to an invaliding report or case book. The form is not so complicated as it may appear at first sight, for every effort has been made by the free use of graphical methods to render results which would otherwise be merely a collection of figures, striking and easy to understand.

Roughly speaking, this form may be divided into two halves. The schema or chart on the left hand side of the paper is intended to be used mainly for chronic cases, and the information noted about height, weight and chest measurements is used by some in such cases. This chart deals with both the quality and the quantity of the urine excreted. The schema on the right hand side of the report form is intended to be used when the analysis is made in a case of acute disease. It may be also employed, to any desired extent, for chronic cases. It illustrates only the quality of the urine.

The specimen report form given has been completely filled in for purposes of illustration. So complete an analysis would only rarely be required. The time spent on this analysis was four and a half hours. Two hours is generally sufficient for an analysis as usually carried out for ordinary cases.

The chart on the left hand side is intended for chronic or convalescent cases ; it is so restricted because an endeavour is here made to estimate the amount of the urinary output for the twenty-four hours, as well as its quality, and to furnish a normal standard for each individual case based on body-weight, and other factors ; these are data not to be obtained generally in cases of acute illness, and would be of doubtful value if they could.

The chart just referred to is adopted from Gautrelet, of Vichy, who introduced it. Gautrelet argued that if the cyclical urine of a certain number of young, healthy and well-proportioned people, living under ideal hygienic conditions, were collected and examined over a period sufficiently long to exclude accidental error, a normal standard of urinary excretion per kilogramme of body-weight could be formed, and that, by calculating the number of active or functional kilogrammes in every individual case, the one amount multiplied by the other would give the normal or ideal urinary cyclical output for the individual concerned. Experiments had been carried out some time previous to this by a French army surgeon, Peyraud, of Lebourne, on the correct proportion of weight to height, &c. Great numbers of healthy young soldiers were examined and the average worked out. Gautrelet adopted Peyraud's figures for his schema, which practically worked out that the weight in kilogrammes would be four-tenths of the height in centimetres, and one and three-fifths the interacromial measurement. The cyclical urine of twelve healthy French peasants of Burgoyne, of both sexes, ageing from 30 to 35 years, fulfilling the necessary requirements of weight to height, and living under almost ideal hygienic conditions as to food, temperature and exercise, was examined for eight days, and the average urinary output for the twenty-four hours per kilogramme of body-weight estimated. This amount, which worked out to 24 cc. of water, 1 gramme of extract, 0·5 gramme of urea, 0·1 gramme of chlorine, 0·005 gramme of phosphoric acid (in terms of P_2O_5) 0·001 gramme of uric acid and 0·001 gramme of urobilin was adopted as the standard or urological unit. The acidity equalled 0·5 cc. normal alkali solution or 0·03 gramme P_2O_5 per kilogramme. One factor in determining the normal standard for the individual had now been acquired, and if quantitative analysis had only to deal with healthy well-proportioned people of from 30 to 35 years of age, living

under healthy conditions, there would have remained only the determination of the other factor or weight. Gautrelet considered, however, that weight alone could not be adopted as the other factor in estimating the normal standard for the average patient who came to Vichy, for he might be very fat and short, and, as fat does not play so active a part as muscle in nitrogenous metabolism, any normal found by multiplying the kilogramme output by the weight would in this case be too high and misleading; or the patient might be thin and muscular, when the opposite would be the case. Again age had to be allowed for, a youth, say, of 15, would not be expected to weigh four-tenths of his height, although this deficiency is somewhat counterbalanced by the greater intensity of metabolism in the young, causing a proportionately high urinary output.

Food, too, which plays so important a part in any calculation of urinary output, had to be taken into consideration. It was with a view to reducing the amount of error from these many sources that the following principles were adopted in estimating the second factor of the two necessary to ascertain the individual normal. A mean is taken, between the actual weight and the theoretical weights for height and chest measurement, with a view to correcting the influence of ill proportion or excessive fat; the influence of age is calculated on the mean or average thus found. Thirty is adopted as the age at which growth has ceased and metabolism become stable, and no allowance made if the patient is of this age; half the difference between the actual age of the individual and 30 is deducted from or added to the height-weight mean which has been found for patients below or above this age. This rule is only followed between the ages of 18 and 45; other rules govern the cases of very young and old persons. The figure resulting from the calculations of height and weight, influenced by age, is the second factor in the attempt to form a normal individual standard; this factor has been called the "biological co-efficient" and represents in an approximate way the number of active kilogrammes of body-weight.

The "individual normal" is then deduced by multiplying the urinary output, per kilogramme, by the biological co-efficient. The amount so found is not affected if the patient is on an ordinary mixed diet, but the result is multiplied by 1·25 if a purely nitrogenous diet is being taken, and by 0·66 if no diet is being taken at all.

I have described this method of Gautrelet's at some length, as it is an ingenious attempt to solve an extremely difficult problem, and because it is the system on which the normals have been found in the schemata, to be given later on, and which are identical with the one on the left hand side of the suggested form of report. The method lends itself to criticism and has obvious defects; but as the same system has been applied to all the cases, the results have probably not been materially affected. To obtain an absolutely accurate standard for each individual is, I am convinced, quite impossible, even if the greatest care were taken in estimating the food intake, body weight, &c., of the individual. We have always to deal with the difference between man and man, that is, the difference of individual metabolic intensity. It is for this reason that I have described the simplest of the two methods given by Gautrelet, for estimating the normal standard. Gautrelet, in a further endeavour to achieve the impossible—an accurate and absolute individual standard—fills the paper with calculations, including a great many body measurements. The method has but to be seen to be condemned as impracticable, while it is doubtful if it is more accurate than the simpler method. Yet it is on a normal so obtained Gautrelet endeavours to dogmatise and draw conclusions from deviations, however slight, from the normal. Bouchard has gone to even further lengths in an endeavour to find an absolutely accurate normal standard, arguing that it is only the fixed albumen of the tissues which is the active agent in nitrogenous metabolism; he seeks to estimate the amount of this in each individual case. Pages and pages of one of the volumes of the last great work on pathology in France are filled with abstruse calculations, allowance being made for, among other things, the bony framework and the skin surface.

The question then arises: Is such a graphic system of illustrating the urinary output, and having for its unit of comparison a normal so open to error, of any value at all? The answer must be decidedly in the affirmative, if the system be applied to the right class of cases, and it be clearly recognised that the results must always be relative rather than absolute, with more attention paid to the relation of the curve of the output to itself than to its position with respect to the normal line. In Gautrelet's system no difficulty exists in finding the urinary output per kilogramme

of weight. The difficulties of the system and its weak points are made evident when the second factor of the normal standard —the biological co-efficient—is estimated. They are as follows: (1) Insufficient allowance made for differences in the urinary output at different ages. In children and very young people the output of urinary constituents per kilogramme is much higher than in the adult, and for these young people it is almost impossible to obtain even an approximate normal standard. (2) The difficulty in estimating and allowing for the influence of excessive fat in the individual; for it is doubtful if such an arbitrary remedy as taking the mean between the actual and theoretical weights really overcomes this difficulty. (3) The insufficient and rather arbitrary allowance made for the influence of food, and the doubt which arises as to whether in private practice, where the system was used, sufficient allowance could be or was made for the food taken by the patients.

It may be opined that few of these difficulties would exist in any application of the system to the soldier, for here all the cases examined would be drawn from young men between the ages of 20 and 30. No special body measurements would be necessary, such as those which have been devised to meet the case of patients who go to Vichy or similar watering places, and who are often very corpulent, and whose weight is not a good index of their active tissue. In other words, for routine army work, physical details other than body weight might be ignored.

In the case of the soldier, the influence of the diet taken could easily be calculated in terms of carbon and nitrogen, as the dietaries are more or less fixed and their nutritive value known. In short, if the urinary output per kilogramme of body-weight were calculated by taking the cyclical urine of a number of healthy men for a period of days, the nutritive value of their diet being known, and the output per kilogramme for twenty-four hours adopted as the standard unit, the normal of any case could approximately be found by multiplying this amount by the number of kilogrammes which the patient weighed. The influence of the food taken by the patient on the result, being allowed for, such a method as this would be a simple one with strictly limited applicability, it could only be used for the soldier whose normal has been determined. The reason it can be used at all is because the men are mainly of an age (20 to 30) when metabolic intensity

does not vary very greatly. I do not think it is possible to devise a satisfactory system for use with children, owing to the great difficulty in fixing a normal. The urinary output per kilogramme varies so greatly at different ages up to 20 that a normal would have to be found for each year of life.

With the modifications above described we could have a simple and approximately accurate method for working out urinary analysis in the army, the graphical method employed rendering the results more striking and interesting, and, above all, we would have some organised system on which to work and compare results, a most important point. The results found would be relative, and greater attention would need to be paid to the shape of the curve of the constituents than to the position of that curve to the normal line. That such a system as has been described is sufficiently accurate will be readily seen when it is shown how the influences of disease on metabolism and on the urinary output are so marked as to overshadow small errors.

The schema on the right side of the report form is easy of explanation. The dark columns denote the normals, while the light double columns show the amounts actually found. It will be noticed that the "normal columns" are broken towards their upper ends; this is intended to show the limits between which the constituent concerned may vary and yet remain in normal proportion, for it is impossible to draw hard and fast lines. This graphic representation would generally be the only one filled in in acute cases, while being filled in to any desired extent in chronic cases.

The rest of the report form presents no difficulty: a description of the physical characters of the urine occupies one corner and the results of the qualitative analysis for abnormal products another, while the microscopical or bacteriological results are suitably represented. Finally, a short space is left for remarks which might be filled in by the person analysing the urine, and be of use to the doctor in charge of the case.

Having, so far, considered general principles, I propose now to give a brief account of the methods employed for the analysis of each constituent of the urine, taking them in the order in which they come in the suggested scheme. Before doing so, however, I would like to draw attention to certain burettes with which most of the work can be conveniently done, and which are shown

in the accompanying photograph. These burettes are self regulating, and are the invention of Dr. Huguet, Professor of Chemistry in the University of Claremont Ferrand in France.

The bottle of the burette is filled with the re-agent to be used. The burette bottles in the photograph are filled with (1) deci-

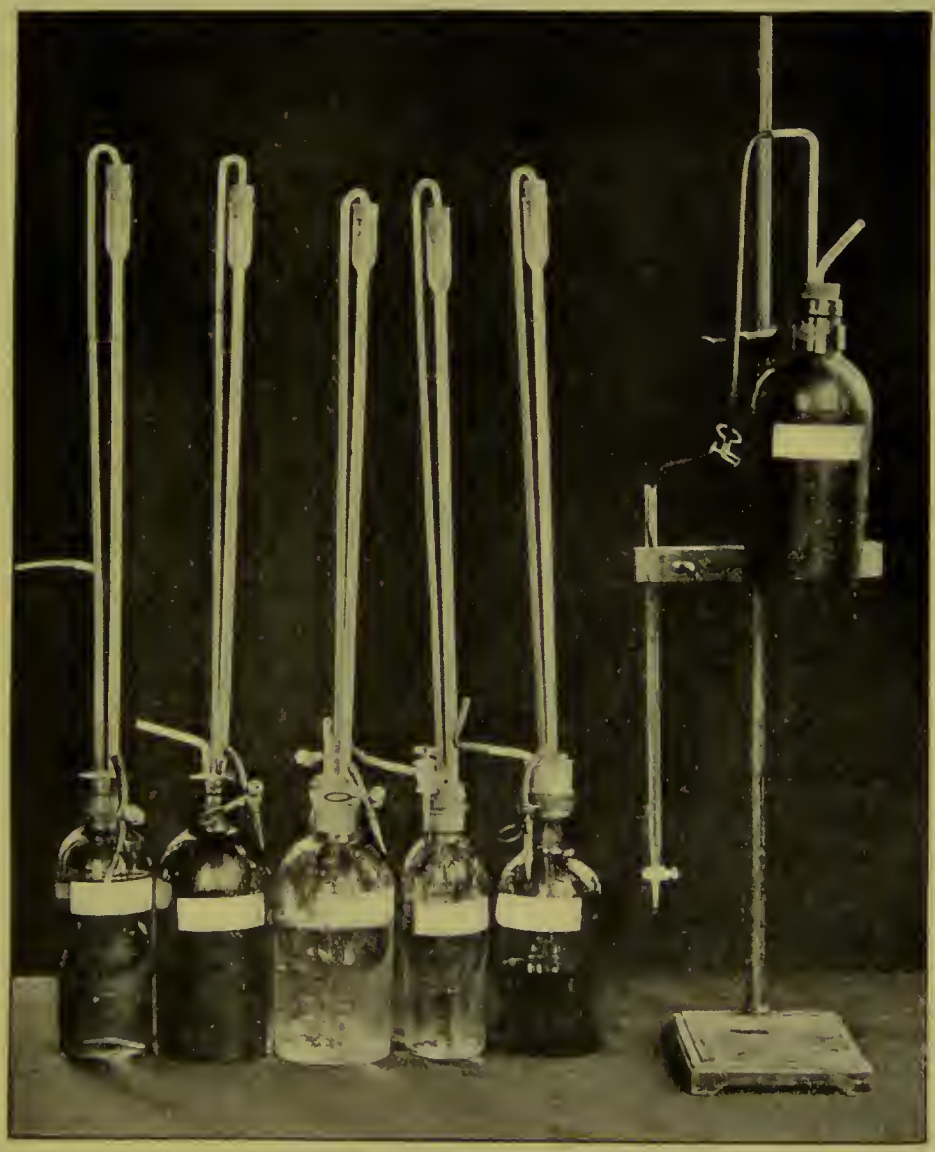


Fig. 1.

normal alkali solution to test acidity ; (2) decinormal nitrate of silver solution for chlorides ; (3) decinormal acid solution for alkalinity ; (4) nitrate of uranium solution for phosphates ; (5) a solution of copper for the estimation of the uric acid.

By blowing down the small tubes seen jutting out horizontally just above the cork, the fluid in the bottle of the burette is forced up through the feeder-tube into the graduated burette tube; by a simple arrangement, syphon action is brought to bear as soon as the fluid reaches the zero mark of the burette, so that the fluid must always remain at this point. The burettes are sufficiently accurately graduated and clearly marked that an amount up to one-hundredth of a cubic centimetre can be read off, if desired. A great deal of time and labour is saved by the use of these burettes, for with them a quantitative analysis of the acidity or alkalinity of the urine, together with the chlorides, phosphates and total purins, can be carried out in half an hour.

The apparatus shown on the right hand side of the photograph is one improvised by means of a bottle and ordinary burette; this gives as rapid results as the Huguet burettes, and can be easily put together.

Notes upon Methods Employed.

I unfortunately do not know some of the methods used by one of the observers (Richard) whose urine charts will be shown in my next paper. But, as a rule, the methods employed have been on similar lines to those about to be described here, and when this is not the case the fact will be mentioned. It will be convenient to consider the methods under the following heads.

Volume.—The urine analysed has always been that of the twenty-four hours—analytical results based on isolated samples are misleading, as the quality of the urine varies greatly at different periods of the day. To prevent decomposition of the urine, a little cyanide of mercury, chloroform or ether is placed in the collecting vessel.

Extract.—In the first six charts this has been estimated by evaporation and drying at 100° C. This method is not absolutely accurate, owing to the volatilisation of certain substances in the extract at this temperature. In charts 7 to 12, and all later work, I have estimated the extract by densimitry; the specific gravity is calculated with great care to four places of decimals and the last two numbers multiplied by 2·33. This gives rapid and sufficiently accurate results.

Acidity.—The acidity of the urine is estimated by means of a decinormal alkaline solution, the process being continued until

an alkaline reaction is given with neutral litmus paper, the results being given in terms of anhydrous phosphoric acid (P_2O_5); this method is the one adopted by Gautrelet. By giving the amount of acidity in terms of phosphoric acid some comparison may be made with the total phosphates. I have latterly also estimated the acidity by Folins' method, which estimates the mineral and organic acidities separately; but this method was not used for all of the charts to be given in the second and further part of my paper. Acidity being the normal condition of the urine, it alone is allowed for in the graphic representation of percentages. Should the urine be alkaline, the alkalinity is estimated by a decinormal acid solution, and the cause of the alkalinity determined.

Total Nitrogen.—This is determined by the well-known Kjeldahl method, the oxidiser used being peroxalate of potassium. Very simple and cheap apparatus can be bought or may be constructed from laboratory apparatus for this method.

If rapid results are required, a Kjeldahl-Henniger process is employed. This consists in neutralising the acid ammonium sulphate formed during the first part of the Kjeldahl process and decomposing the neutral ammonium sulphate in a ureometer, instead of distilling it over into a decinormal acid solution as is usually done. The Kjeldahl-Henniger process gives accurate results only if great care has been taken not to render the ammonium sulphate alkaline, or to prevent the great heat evolved during the process by keeping the vessel used in the process in cold water.

Urea.—A considerable amount of time has been devoted to endeavours to find a rapid and accurate process for the estimation of urea. Nearly all the better-known methods have been tried and rejected either because they were inaccurate (Liebig's method and its modifications and the unmodified hypobromite process), or because they were long and complicated (Moerner, Braunstein, Folins, and Bohland's methods) and had no clinical value. The hypobromite method is by far the simplest of all urea processes, and eminently suited for clinical work; but owing to the manner in which it is almost invariably carried out in England it is so inaccurate as to be worthless. Earlier in this paper I expressed several reasons why this process was inaccurate, but as the method I have adopted, and am about to describe, is based on the hypobromite process, I may recapitulate them.

(1) The nitrogen of other bodies beside the urea is liberated

by the hypobromite solution. In a number of experiments carried out by reacting on solutions of some of these bodies with hypobromite, the following results were obtained: Uric acid evolved from 25 to 45 per cent. of its nitrogen, kreatin 50 to 70 per cent., kreatinin 30 to 40 per cent., and ammonia salts gave up all their nitrogen in contact with the hypobromite, while no gas could ever be obtained from hippuric acid. The smaller percentages quoted in each case were obtained when no glucose had been added to the solution under examination, while the higher percentages were obtained by the addition of glucose, and by violent agitation of the hypobromite and experimental solutions. These results are not dissimilar to those obtained by Falck and other observers.

(2) No allowance is generally made for temperature, which so greatly affects the bulk of gases.

(3) The use of bad ureometers and the want of care in the preparation of the hypobromite solution. The method adopted, which retains the use of hypobromite and at the same time overcomes the objections just mentioned, is as follows: All the nitrogenous bodies of the urine, except the urea, are precipitated by a solution of phosphotungstic acid (in excess), acting on a urine acidulated with hydrochloric acid. To 20 cc. of urine are added 2 cc. of a 10 per cent. solution of hydrochloric acid, and the mixture made up to 60 cc., with a 9 per cent. solution of phosphotungstic acid. The mixture is allowed to stand for half an hour and is then filtered; 15 cc. of the filtrate, which represents 5 cc. of urine, is reserved for use with the ureometer.

Sallerin, Donzé and Lambling have shown that uric acid, kreatin, kreatinin, the xanthin bodies, and ammonia salts are precipitated from the urine by phosphotungstic acid, and though I have been able to recover minute traces of ammonia from a phosphotungstic filtrate by Shaffer's method, this does not militate against the fact that for practical purposes the ammonia is completely precipitated. As phosphotungstic acid in greater strength than 11 or 12 per cent. precipitates small quantities of the urea, 9 per cent. has been fixed upon as a convenient strength, and by using two volumes of the acid to one of urine one can always be sure of having an excess present.

Fifteen cc. of the filtrate are placed in one of the legs of the 人 shaped tube attached to the ureometer in fig. 2, and a solution

PLATE I

REPORT OF URINARY ANALYSIS.

NAME—J. S.
DISEASE—Chronic Dyspepsia.
AGE—30. WEIGHT—65 Kilos. HEIGHT—1 Metre ·70.
INTERACROMIAL MEASUREMENT—·39 Metre.
ANTERO-POSTERIOR DIAMETER OF CHEST—·21 Metre.
(3) BIOLOGICAL COEFFICIENT—67.
DIET—Mixed, Full.
TREATMENT (Drugs)—Nil.

PHYSICAL CHARACTERS OF URINE.

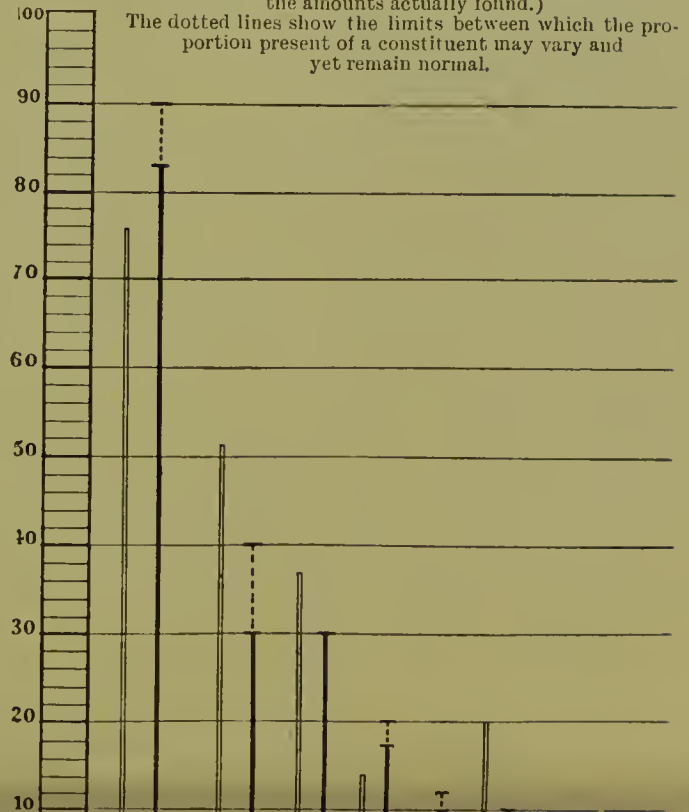
COLOUR—Citron Yellow. DEPOSIT—Flocculent (Mucus).
ASPECT—Limpid. ODOUR—Sui Generis.
REACTION—Acid. DENSITY AT 15°C—1014.
POLARIMETRIC DEVIATION—1° of Laurent's Polariscope.
POINT OF CONGELATION—Not Taken.

(2) CHART OF URINARY OUTPUT OF THE PATIENT FOR THE 24 HOURS.

(And the percentages of the constituents to normal.)

CHART OF RATIOS OF URINARY CONSTITUENTS.

(Black columns denote the normal, double columns the amounts actually found.)
The dotted lines show the limits between which the proportion present of a constituent may vary and yet remain normal.



Found in 24 hours	1980	65·54	2·65	8·63	28·39	·61	2·33	·80
Normal for the 24 hours	1610	67	2·01	6·70	30·15	·67	3·55	·67
Percentage to Normal (100)	125	96	133	127	93	91	68	120

CHART 1.

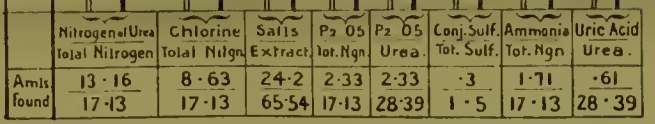
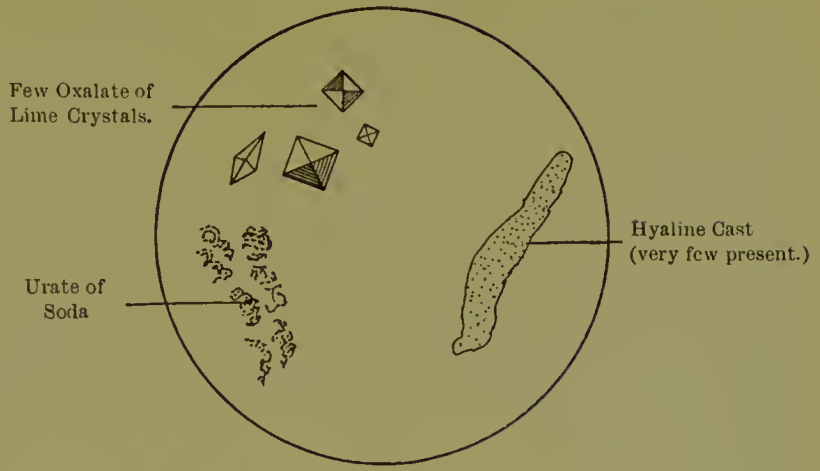


CHART 2.

QUALITATIVE EXAMINATION.—ABNORMAL PRODUCTS.

ALBUMEN { Globulin. / Serin—Traces.
ALBUMOSES—Traces.
PEPTONES—Nil.
BILE SALTS—Nil.
B. PIGMENTS—Nil.
SUGAR—1 qr. in 24 hrs.
ACETONE— / DIACETIC ACID— / β OXYBUTYRIC ACID— } Nil
INDICAN—Increased.
SKATOL—Increased.

MICROSCOPICAL AND BACTERIOLOGICAL EXAMINATION.



REMARKS.

The analysis shows the condition of the patient to be mainly hepatic and gastro-intestinal. The high urinary acidity and urobilin present point to the liver being affected (functional insufficiency), while the increased proportion of conjugated sulphates and particularly skatol and indican indicate excessive intestinal putrefaction. The increased ratio of ammonia present is probably due to an increased absorption of acid products from the gastro-intestinal tract, needing neutralisation, and diverting the ammonia from its normal course, viz., to form urea. The small amount of sugar present is due probably rather to hepatic insufficiency than to any more vital cause. The presence of traces of serin and albumoses and a few hyaline casts is possibly due to nutritional disturbance temporarily affecting the kidney and not to organic kidney disease.

NOTE.—1. Metric weights and measurements are used in this report. 2. The chart on the left hand side of this report is intended to show the quantity as well as the quality of the urine, and is only filled in in chronic or convalescent cases, the information concerning height, weight, &c., being necessary only in such cases. The normal used in this chart varies with the individual; it is found by multiplying the output which each healthy kilogramme of body weight is known to excrete by the biological coefficient. 3. The biological coefficient is found by taking the mean between the actual weight and the theoretical weights for height, and chest measurement, age, diet, are also allowed for.

ERRATUM.—For ratio $\frac{\cdot 3}{1\cdot 5}$ in the $\frac{\text{Conj. Sulph.}}{\text{Tot. Sulph.}}$ column read $\frac{\cdot 59}{2\cdot 95}$. The former ratio is relatively correct, but represents the amounts of sulphates found *per litre*, and not for the *total* urine (1980 c.c.).

of hypobromite of soda placed in the other. The gas evolved when the two solutions are mixed is read off and noted when contraction has ceased. This result, when compared with the amount

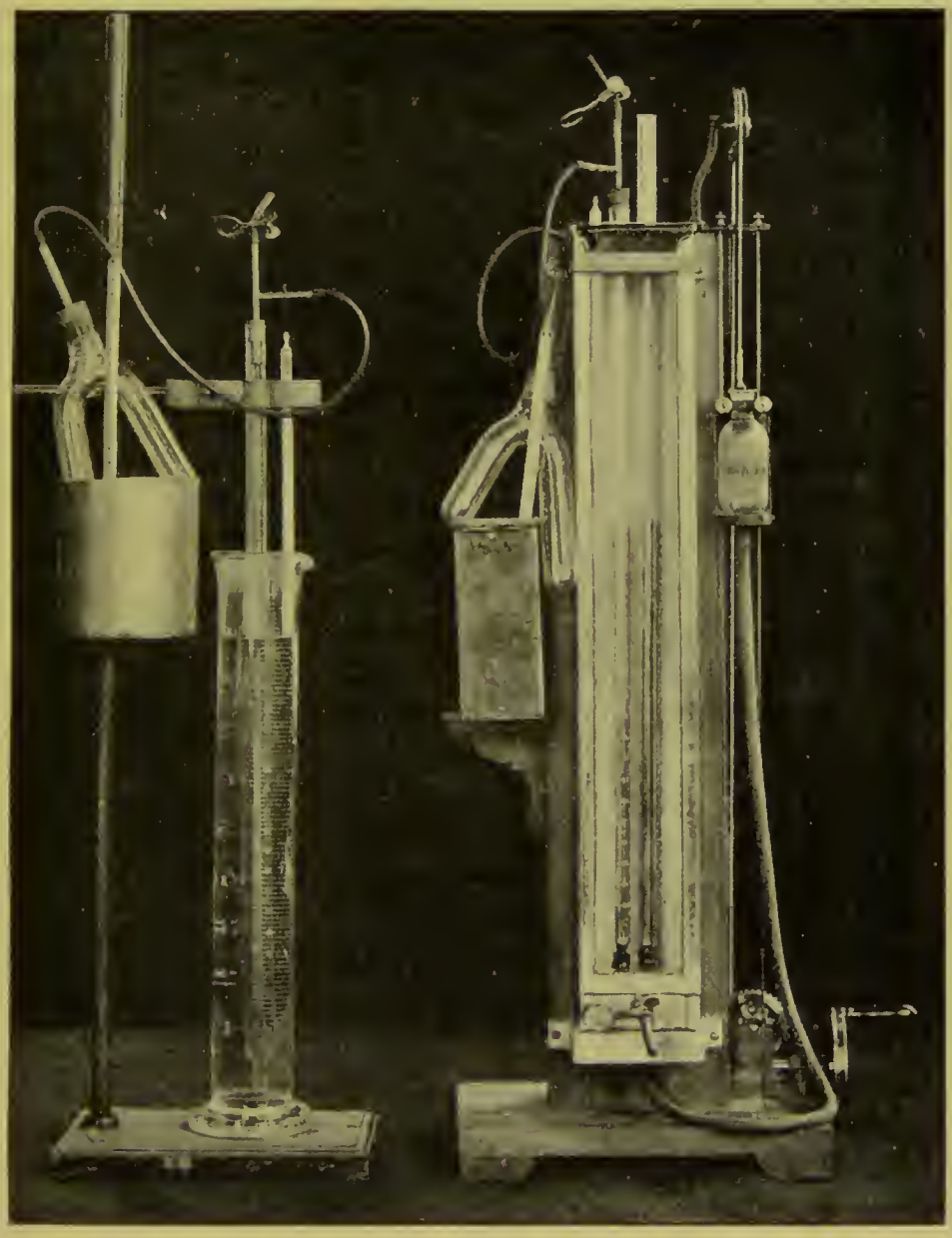


Fig. 2.

of gas given off by 5 cc. of a 2 per cent. solution of pure dried urea—under exactly similar conditions of temperature—gives the amount of urea per litre in the urine.

In the accompanying fig. 2 will be found illustrations of the

two ureometers used in the urea estimations. The one on the right-hand side of the figure has been constructed by joining two burettes together with a U shaped tube and connecting the lower arm of the same tube to a movable reservoir of mercury by means of a piece of rubber tubing. The ⅄ shaped vessel in which the reaction between the hypobromite and urine solutions takes place is attached to the upper end of one of the burettes by a ⊢ shaped tube and a piece of rubber. Both the ⅄ shaped reaction tube and the burettes are placed in water boxes containing thermometers. The ureometer just described has been placed on the stand of a Mereier's instrument ; but a simpler stand can be easily made.

The ureometer on the left of fig. 2 is of simple construction, and has been made up out of laboratory apparatus. It needs no explanation.

The advantage of ureometers such as these is that all the gas-holding apparatus is surrounded by water which can be brought to the same temperature before the gas, evolved by (1) the urea solution or (2) the urine, is read off. The temperature of the gas being the same in both cases, no allowance need be made for it, thereby avoiding troublesome calculation.

I have given up using glucose—which increases the amount of nitrogen evolved by the urea—except in diabetic urines—and although only 92 per cent. of urea nitrogen is evolved by hypobromite alone, this does not influence the result, which is a comparative one between a pure urea solution and the urine examined. Glucose by evolving heat complicates matters.

The hypobromite solution is prepared by adding equal parts of a 40 per cent. solution of caustic soda and a mixture containing 10 cc. of bromine dissolved in 100 cc. of a 15 per cent. solution of bromide of sodium. This is used only when freshly prepared.

The urea process just described takes twenty minutes to complete (excluding the time taken for precipitation), it is simple of execution, rapid and accurate. In my hands it has given very satisfactory results in a long series of estimations.

Chlorine.—This is estimated by Mohr's process as modified by Pribram. The modification consists in the destruction of urinary organic matter, which otherwise combines with the silver nitrate solution and vitiates the results. The organic matter is got rid of by heating with sulphuric acid and permanganate of potassium.

Uric Acid.—This is estimated by one of the observers (Gautrelet) by his modification of Arthand and Butte's method, the re-agent used being a hyposulphite of copper, and the indicator ferrocyanide of potassium with hydrochloric acid. The process is simple, very rapid, and gives fairly accurate results. It is the method used in some of the charts to be described.

The uric acid estimations shown in the charts of my own cases have been done by Denigès' method, which has been adopted

Fig. 3.

as the standard method in France, and for which very accurate results are claimed. Copper hyposulphite is the re-agent used, and the amount of uric acid in the urate of copper which is formed is estimated by means of a cyanide.

Phosphates.—These are estimated by the nitrate of uranium method, the indicator used, however, being that recommended by Mercier, viz., tincture of cochineal, which appears to give better results than the ferrocyanide of potassium drop method.

Urobilin.—This pigment is estimated spectroscopically, the instrument used being Gautrelet's uropigmentometer. This instrument is illustrated in the accompanying photograph (fig. 3), and consists of a spectroscope held vertically into a glass vessel filled with the urine to be examined and placed on a stand. By turning a screw on the instrument, this vessel can be raised or lowered at will, thus decreasing or increasing the depth of the layer of urine examined; the movement which raises or lowers the glass vessel also turns a disc at the bottom of the stand, which automatically registers the depth of the liquid thus produced. As soon as the spectrum of urobilin—a dark absorption band near F—is seen, the depth of the layer of urine is noted. A calculation table accompanies the instrument, from which, according to Gautrelet one can estimate the amount of urobilin per litre.

A good deal of the urobilin of the urine when freshly emitted exists as a colourless chromogen, and it is sometimes almost impossible to detect the characteristic spectrum in such a case. On exposure to the air, however, this chromogen becomes converted into urobilin, completely according to some, partially so according to others. In the urine of twenty-four hours a band can always be seen by anyone used to spectroscopic work. To intensify the absorption band, Denigès, of Bordeaux, employs a solution of iodine in iodide of potassium, which is supposed to act by oxidising the chromogen to urobilin. I have adopted this method latterly, using 1 cc. to 100 cc. of urine. In the charts, however, to be given, the urobilin has been estimated directly.

Ammonia.—This is estimated by Shaffer's method. This method consists in driving off the gas by boiling it with an alkali *in vacuo*, the boiling point, considerably lowered by the condition of vacuum, being further reduced by the addition of methyl alcohol to the urine. The mixture thus formed boils at from 40° to 45° C., and as urea is not broken up till a temperature of 60° C. is reached, the results are not liable to be vitiated by ammonia so derived. The accompanying photograph illustrates the apparatus (fig. 4).

The boiling is carried out in a water-bath, and the small bulbs are used to prevent the alkali being carried over to the small flasks which contain the decinormal solution of sulphuric acid, into which the ammonia is received. The amount of ammonia present is estimated by the loss in acidity of this decinormal acid: each cc. of acidity being equal to 0·0017 gramme

of ammonia. The vaeuum is produeed by the use of an inexpensive water-suetion tube, the degree of vaeuum produeed being shown by the large U-shaped pressure-tube placed on the

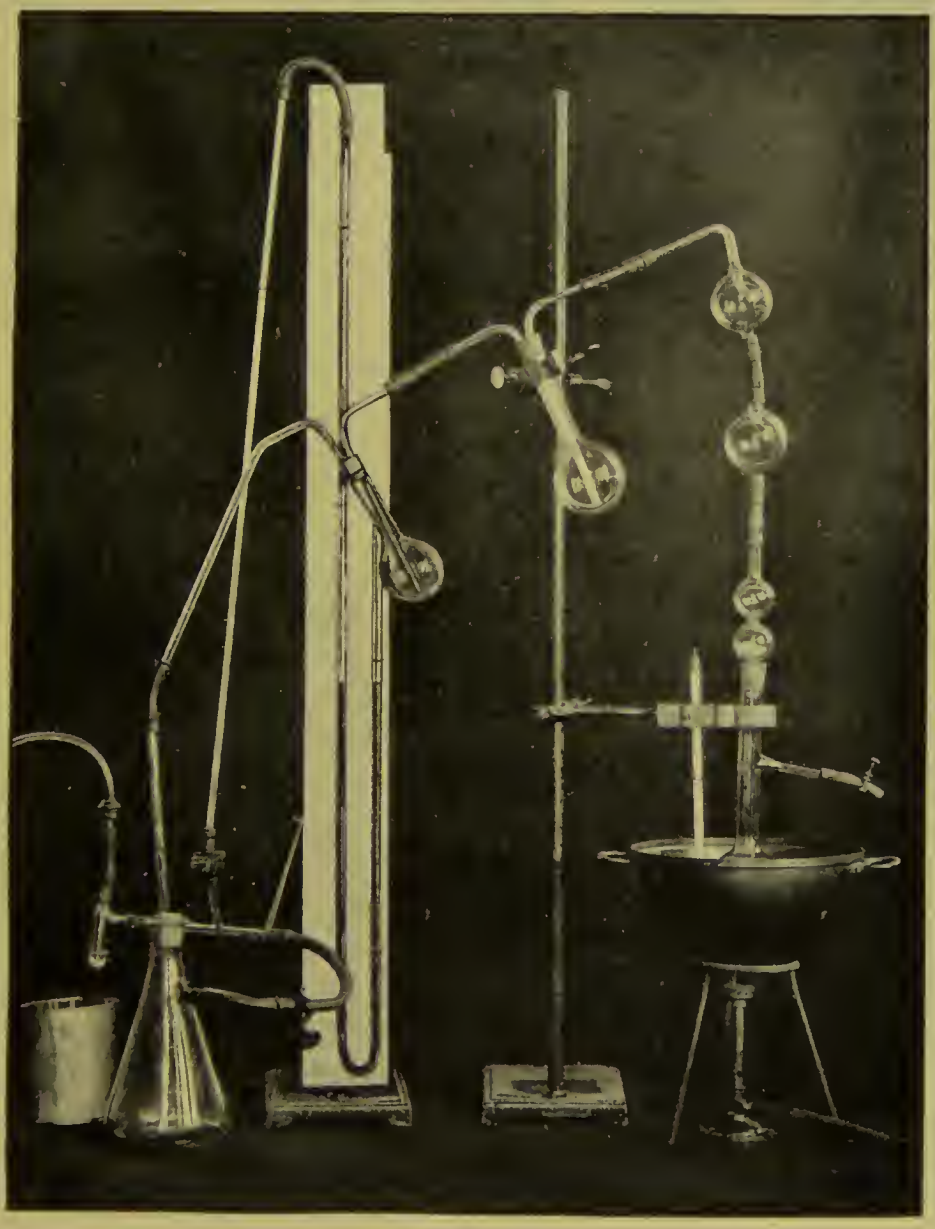


Fig. 4.

white millimetre eard (the process should be carried out at a pressure of 10 millimetres of mereury). The apparatus thus set up is simpler than it may appear at first sight; it is inexpen-

sive and gives very accurate results ; it can be fitted up anywhere if a sufficient pressure of water can be assured. The apparatus shown in Shaffer's description does not given very satisfactory results—the one in the figure has been modelled on one set up by Dr. Beddard in the Guy's Hospital laboratories, and I owe it to his advice that I have escaped the inevitable trouble inseparable from work with new processes.

Sulphates.—These are estimated by Salkowski's gravimetric method.

Some of the methods employed for the qualitative and quantitative analysis of abnormal products in urine which occupy a place in the report form may be briefly referred to. *Albumin* is estimated quantitatively by the gravimetric method, though, if a Purdy's centrifugaliser be present, the centrifugal method is greatly to be preferred. Qualitatively, Borreau's reagent—sulphophenic and sulphosalicylic acids—has been used in addition to the ordinary tests, as it is so delicate. *Sugar* is estimated by Gerard's cyano-cupric method. *B-Oxybutyric acid* by the polariscope, *i.e.*, by reading the polarimetric deviation caused by the substance in solution *plus* the sugar ; the quantity of the latter being known and also its effect on polarimetric deviation, the amount of B-oxybutyric acid can be readily calculated by difference.

The methods adopted for the albumoses, peptones and acetone, and the qualitative tests for albumin, sugar, bile acids and pigments are those usually adopted and described in all text books on urine. It will be noticed that with two exceptions the processes noted as being used are rapid processes. These exceptions are the gravimetric methods of estimating the sulphates and albumin. Purdy has shown conclusively, however, that with carefully arranged centrifugal methods both the sulphates and the albumin, can be estimated with great rapidity, and with an accuracy quite sufficient for all clinical purposes. I hope in future work to be in a position to employ these methods. The ratio of the constituents to each other, as shown in the schema of columns and the percentage amounts of the constituents of the urine to the calculated normal, are easily found, once the amounts actually present have been estimated.

For further information about the procedures mentioned, notably the methods of estimating the acidity and alkalinity of the urine, and the amount of chlorides, phosphates, sulphates,

sugar and albumin, any standard work on urinary chemistry may be consulted; but for the information of those interested in this subject, I submit a short bibliography. The practical application of these methods in actual cases of disease, with comments upon their values, will be given in a subsequent communication.

(For Bibliography see pages 63 and 64.)

CLINICAL URINARY ANALYSIS.

A Critical Study.—Paper II.

(*Reprinted from the "R.A.M.C. Journal" of February*, 1904.)

In the September number of this Journal appeared a paper on this subject in which I described a suggested form of report for quantitative urinary analysis, and also devoted some space to brief notes on the methods employed in its compilation. In this paper will be given some schemata or charts based on actual cases, for the purpose of illustrating the clinical value of a system of careful urinary analysis. The paper will be concluded by remarks on the inferences which may be drawn from variations from the normal in the proportions of urinary constituents, and their import in the prognosis or diagnosis of disease.

To obviate references, the following brief *résumé* of my earlier article is given.

A description was given of an analytical report form for urinary work, which, besides giving the qualitative analytical results and illustrating the microscopical, included two schemata or charts. The first of these charts was intended to show the curve or tracing of the urinary output for the twenty-four hours. The normal for the individual was first found by multiplying the amount of urinary constituents—which an average kilogramme of healthy tissue had been found experimentally to produce—by the number of kilogrammes of body weight of the individual in question. The normal when found was shown by a line drawn at 100, the curve or tracing representing the amounts actually present, being worked out in percentages to the normal. This form of schema is illustrated in charts 3 to 6. The other schema described was one where the quality alone of the urine was considered, the proportions in which the principal urinary constituents were present being shown by a series of columns. The proportions actually found were illustrated by double columns, while the normal proportions or ratios, which served as a guide, were represented by black columns placed alongside the others. Cases worked out on this system are illustrated by charts 7 to 12, plates 4, 5 and 6.

PLATE II.

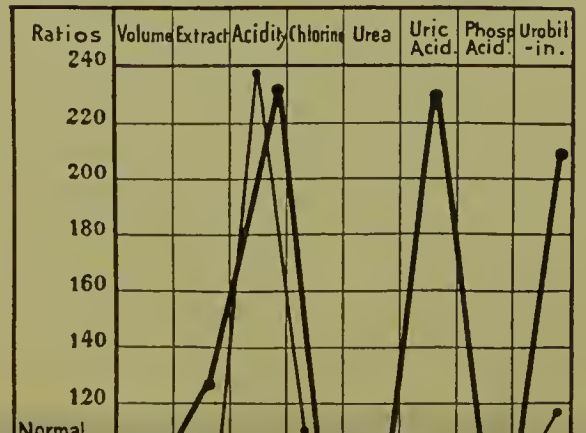
Tracings of Cases of (1) Alcoholic Cirrhosis and (2) Dyspepsia worked out on the percentage schema. The "normal line" at 100 represents the normal urinary output of the individual, the amount actually found being calculated in percentages to that normal. Thus the normal output of A (chart 3) is not that of B (chart 3), but the tracings or curves actually found are in relation to their individual normal. For instance, the volume of urine passed by both A and B was about 1,300 c.c., but while this amount was 95 per cent. of the amount A *should* pass, it was only 68 per cent. of the normal amount of B.

More attention should be paid to the *shape* of the tracing than to its relation to the normal line owing to the somewhat arbitrary manner in which the normal must perforce be calculated. Thus in case B (chart 3) we can see that the urea is less than normal while the uric acid greatly exceeds it, but are more impressed when we realise how greatly the ratio (40 to 1) of the urea to uric acid must be altered in this case (it was actually only 11 to 1).

These first four charts on Plates II. and III. show tracings obtained by different observers, who have not always used identical methods of analysis. Further the diets of the cases compared on the same chart are not *exactly* alike. Although, therefore, an effort has been made to reduce the margin of error, due to difference in diet, by selecting such cases for comparison as were on *similar* diets, and a further attempt made to obtain uniform conditions by using the same (Gautrelet's) normals for all the cases, the results are, I admit, only comparative to a certain degree. It was deemed advisable, however, in spite of the difficulties and possible fallacies just mentioned, to illustrate the work of various observers on a few cases rather than those of one observer on a greater number. By this method perhaps, greater weight is given to the contention that certain diseases appear to affect nutrition in a specific manner.

The amounts actually found in each case have not been given as they take up too much space and would not simplify matters.

TWO CASES OF HYPERTROPHIC ALCOHOLIC CIRRHOSIS.



THREE CASES OF DYSPEPSIA.

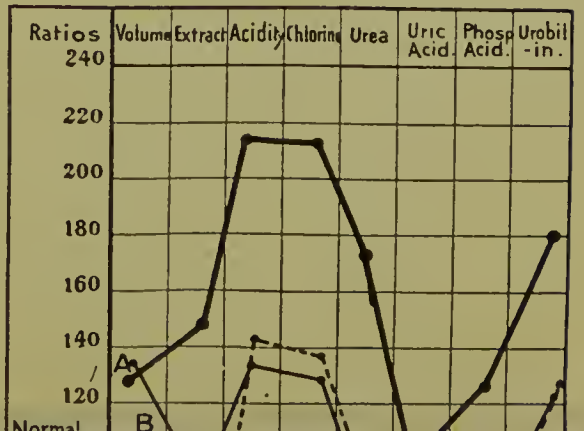


CHART 3.

A. Case of hypertrophic alcoholic cirrhosis from Dr. Dalton's wards, King's College Hospital. This was a typical case. The liver greatly enlarged and tender; the patient appeared jaundiced (though no bile pigments could be found in the urine). The normal for this patient was somewhat arbitrarily determined as he was not weighed, and so the shape of the curve is more important than its relation to the normal line. Qualitative examination —No albumen or sugar; indican in excess. Diet chiefly milk.

B. M. W., a case analysed by Dr. Gautrelet and given in his work "Spectroscopic Critique des pigments urinaire normanx." This was a typical case of alcoholic cirrhosis with an enlarged liver and ascites; traces of albumen, and a marked reaction for indican were found qualitatively. Diet light farinaceous.

REMARKS.

The shape of these tracings with their triple "cones" of high acidity, uric acid, and urobilin is typical of that found in most cases of liver diseases, especially alcoholic cirrhosis. Case B, where several analyses always showed this kind of tracing, well illustrates the important part the liver plays in metabolism. The uric acid is high here because of (1) the increased metabolism of hepatic cell nuclei, and (2) the inability of the uroproteic ferment of the liver described by Richet to convert uric acid into urea as happens in health. The urea found is less than normal because of reason (2), and also because, owing to the inability of the liver to burn up organic acids absorbed from the gastro-intestinal tract, the ammonia which ordinarily goes to form urea is robbed to neutralise them. The organic urinary acidity was found by Folin's method to be increased in this case, and the ammonia N. reached 10 per cent. of the total nitrogen instead of 3 per cent., its normal amount. The increased amount of urobilin present in both cases is characteristic of similar hepatic conditions. The increase is due (1) to the conditions being more favourable for increased hæmolysis and the reduction of the hæmatin and biliary bilirubin to urobilin; (2) to the fact that the impaired efficiency of the liver prevents it destroying the excess of urobilin taken up from the intestinal tract, where it is largely produced by bacterial action on the bilirubin. The effects of intestinal putrefaction on urobilin excretion are well shown in chart 8.

CHART 4.

A. M. F., a case of dyspepsia with diminished secretion of free HCl. Patient is constipated. Liver very slightly enlarged, no other symptoms. Qualitative examination showed abundant oxalate of lime crystals in the sediment. There were traces of albumoses present and the indican reaction was marked. (Case taken from Dr. Gautrelet's "Spectroscopic Critique," &c.)

B. and C. Two analyses made on the same subject. B by Richard, of Nice, at the instance of Dr. Gilchrist, and C by myself, —an interval of four months separated the two analyses. The case was one of chronic dyspepsia with some diminution of free hydrochloric acid in the gastric juice. The symptoms present were flatulence and constipation, the liver was slightly enlarged and a little tender to deep pressure. The appetite was good and a fair quantity of food taken. The patient was somewhat neurasthenic and suffered from occasional attacks of urticaria. Qualitatively, small quantities of sugar, albumen and albumoses were present, with an abundant sediment of oxalate of lime crystals. The reaction for indican was marked, and the ratio of conjugated sulphates to the total sulphates above the normal—(20 per cent. on the second analysis). The ammonia found on the second analysis (C) was 7 per cent. instead of 3 to 4 per cent., the normal amount. Diet on all three occasions mixed ordinary.

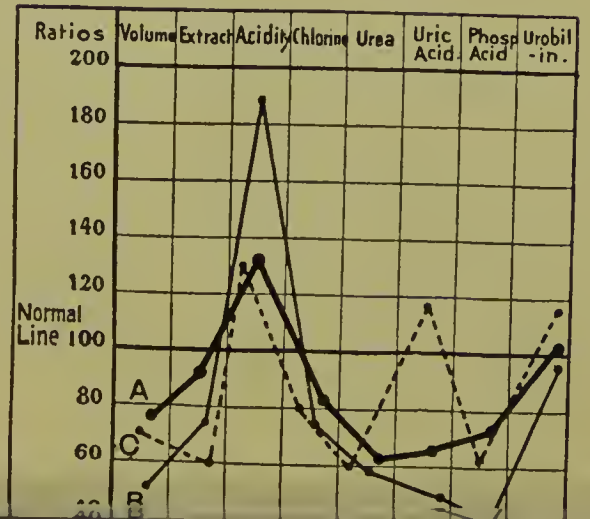
The organic acidity by Folin's method was greater than the mineral acidity. Total acidity 10, organic 6, and mineral 4, in terms of $\frac{N}{10}$ alkaline solution is 25 c.c. of urine.

REMARKS.

This type of tracing is often seen in chronic dyspepsias associated with increased gastro-intestinal fermentation. The high acidity seen in all these cases is due in all probability to the increased absorption of organic acids from the gastro-intestinal tract, and this is proved by the high organic acidity found ($\frac{6}{10}$ of the total acidity). The urea is either actually or relatively low in all these tracings, the reason in case C being the deviation of the NH_3 from its urea-forming function, to neutralise the acidity. This is probably what has also happened in cases A and B. The high urobilin present is due to the increased intestinal putrefaction and also to the liver insufficiency which so often accompanies dyspepsia.

PLATE III.

THREE TRACINGS OF GOUT, TWO REPRESENTING THE TRACING USUALLY SEEN IN THE STATIONARY PERIODS OF THE DISEASE, WHILE THE THIRD, C, SHOWS THAT FOUND TOWARDS THE END OF AN ACUTE ATTACK.



TWO TRACINGS OF TUBERCULAR DISEASE, ONE OF THE BLADDER, A, AND ONE OF THE LUNG, B.

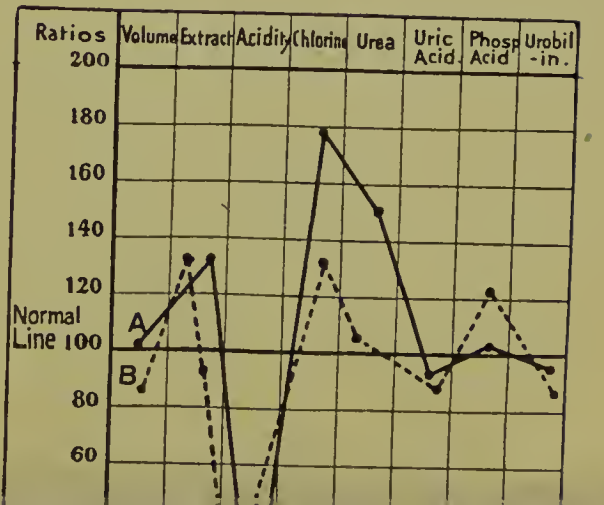


CHART 5.

A. A tracing of a patient S., a case of hereditary gout. There was a gouty family history of generations. The patient is young (30) and a good liver, inclined to be florid and stout. No symptoms of gout yet appeared. Qualitative examination showed traces of albumen and albumoses. Analysis made by Richard, of Nice, at the instance of Dr. Gilchrist. Diet mixed, ordinary.

B. Analysis made by myself of G. M., a typical case of gout. Patient stout, has tophi in ears, and is subject to occasional attacks of articular gout (great toe joint). The analysis was made in the interval between the attacks. Qualitatively slight albumen and few casts present. Diet mixed ordinary.

C. S. J., a case of acquired gout, the analysis was made at the end of an acute attack of articular gout (great toe joint). Qualitatively, albumen present in traces, bile pigments and a few hyaline casts. Analysis made by Richard at the instance of Dr. Gilchrist, of Nice. Diet light farinaceous.

REMARKS.

The tracings A and B illustrate what is generally found in cases of stationary gout or in those who have a gouty tendency, while having no actual symptoms. High urinary acidity and everything else "below the line." Tissue metamorphosis and the metabolic exchange seem to be diminished in gout, there being a tendency rather to put on weight. In tracing C will be found the appearances generally seen in a tracing taken towards the end of an acute attack. There is a "flushing" out of the uric acid which had up till then been defectively eliminated.

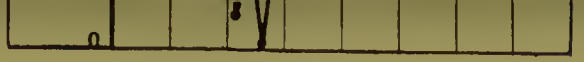


CHART 6.

A. M. W., a case of early tubercle of the bladder in a lad with a family history of tubercle. There was slight cystitis and some renal pain. Tubercle bacilli found in urine, pus present in small amount. Diet mixed ordinary. (A case of Dr. Gautrelet's).

B. G., a case of phthisis, with a small cavity in the right upper lobe. No marked cachexia present. Evening temperature in axilla generally normal, but rises sometimes to 99 or 100°. The analysis was made by myself before any active treatment had been commenced. Diet mixed ordinary.

REMARKS.

Although taken from two cases which differed considerably clinically, there is a strong resemblance between these two tracings. This type of tracing is often met with in cases of early tubercular disease, before treatment has modified the tracing. A chart such as this may also sometimes be seen in those free from tubercular disease, but with a strong hereditary tendancy. The condition is shown by an increased output of solids, especially mineral matter, chlorine and the phosphates being present in increased amounts. The acidity is almost invariably low. These facts may be accounted for by the increased tissue metamorphosis present in tubercular disease, especially lung tubercle, and the more intense respiratory exchange. (Robin, Binet.)

GENERAL REMARKS.

The striking contrast between these two sets of cases, the gouty and the tuberculous, is well shown in these schemata. In the case of gout the lower output of solids is evidence of a diminished metabolic exchange, and as a matter of fact, that tissue construction generally exceeds tissue waste is shown by the tendency of gouty patients to put on weight. The increased output of urinary constituents in tuberculous diseases is a symptom of the metabolic activity induced by the disease. An interesting point is that in a healthy man a purely vegetable diet produces a tracing akin to the tubercular chart, while a purely meat diet tracing is very similar to that seen in gouty cases. A tuberculous patient put on the full meat meals accompanying the open-air treatment acquires the hyperacid tracing. That the gouty subject is usually immune to tubercle is becoming generally recognised, and one is inclined to wonder if the success of the Nordrach treatment may be due to the production of a body condition, a *terrain* like that of gout, which is unfavourable to the well-being of the bacillus.

PLATE IV.

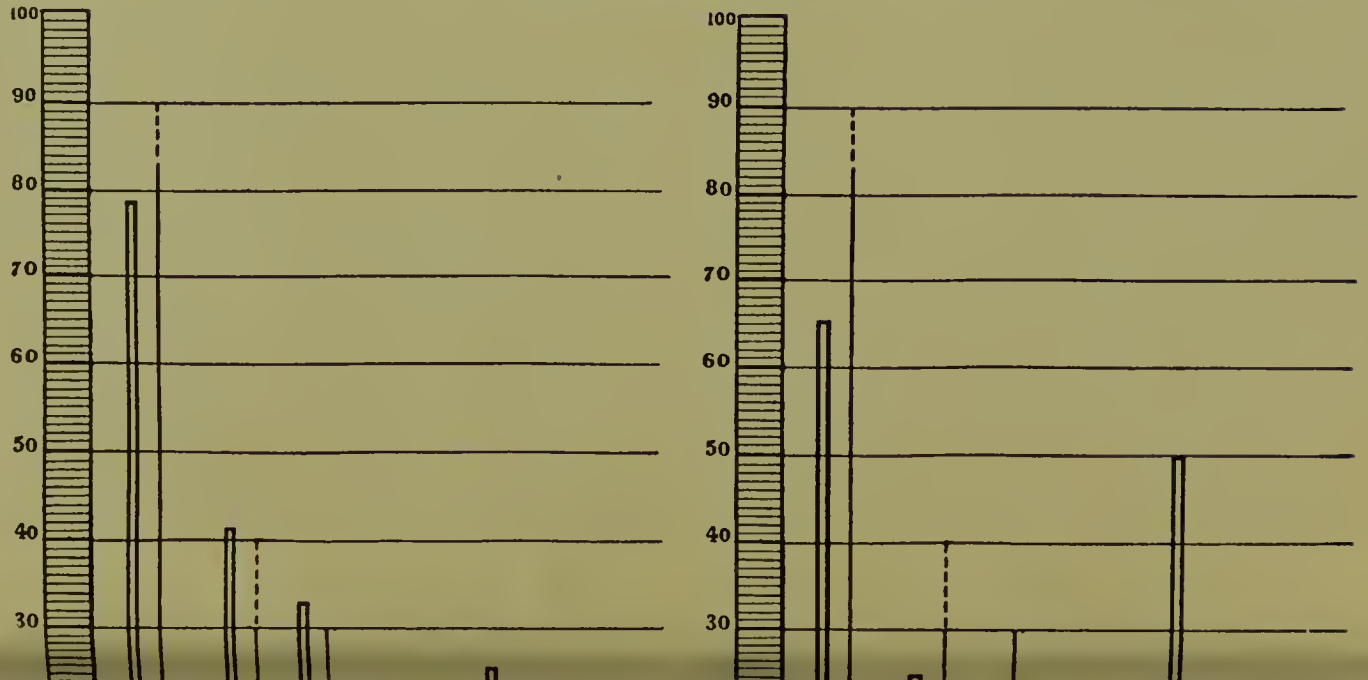
A series of cases worked out on the schema of columns. This schema, which shows the quality of the urine, may be suitably employed in cases of acute disease. In healthy people on ordinary diet there is a ratio of relation between the various normal urinary constituents. In pathological conditions these ratios depart, sometimes widely, from the normal. This schema has been designed to contrast the actual with the ideal. The black columns represent the normal ratios—the dots representing the limits within which the ratio may vary and yet remain normal. The double columns denote the ratios actually found in the case.

A CASE OF CHRONIC DYSPEPSIA. ANALYSIS MADE THE DAY PRECEDING AN ATTACK OF URTICARIA.

CASE OF HYPERTROPHIC ALCOHOLIC CIRRHOSIS.

THE NORMAL RATIOS ARE ILLUSTRATED BY THE BLACK COLUMNS. THE DOTTED TERMINATIONS SHOW THE LIMITS WITHIN WHICH THE RATIOS MAY VARY AND YET REMAIN NORMAL. THE DOUBLE COLUMNS REPRESENT THE RATIOS ACTUALLY FOUND.

Amounts are given in grams.



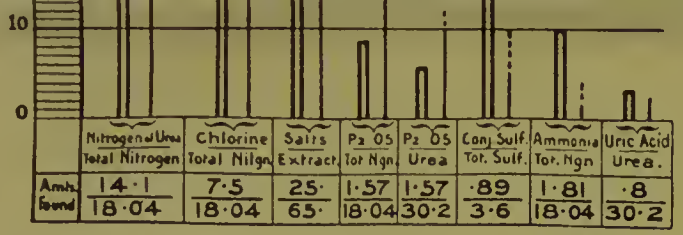


CHART 7.

J. A case of chronic dyspepsia. The symptoms were flatulence and frequent attacks of constipation. Patient anæmic, suffers from occasional attacks of urticaria. This analysis was made the day before an attack of urticaria, which yielded to a smart saline purge followed by a short course of alkaline table waters. Qualitatively, nothing abnormal was found. The acidity was high, 45 c.c. N. alkaline solution per litre. The orgauic acidity was $\frac{6}{10}$ of the total acidity. (Folin's method.)

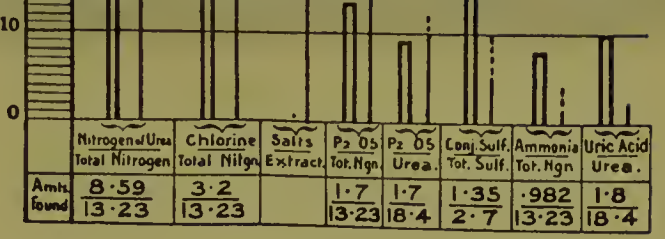


CHART 8.

This is the same case as that shown on fig. 1 (tracing A) a typical case of hypertrophic alcoholic cirrhosis, the liver enlarged and tender, some ascites. Patient jaundiced-looking, but no bile pigments found. The analysis represented bere was made on a different day to that shown in fig. 1 and is given to show to wbat extreme limits the ratio $\frac{\text{conjugated sulph.}}{\text{total sulphates}}$ may rise. I am informed by Dr. Lee, Dr. Dalton's house physiciau, tbat there was no leucocytosis in tbis case.

REMARKS.

Both these schemas well illustrate the point to which I drew attention in my first paper, viz., that the urine might still be abnormal when the qualitative tests for albumen and sugar had been negative. Here are two cases which would be described in a case book as "normal," and it is left to a careful quantitative examination to show how abnormal they are, and how gravely affected the metabolism is in each case.

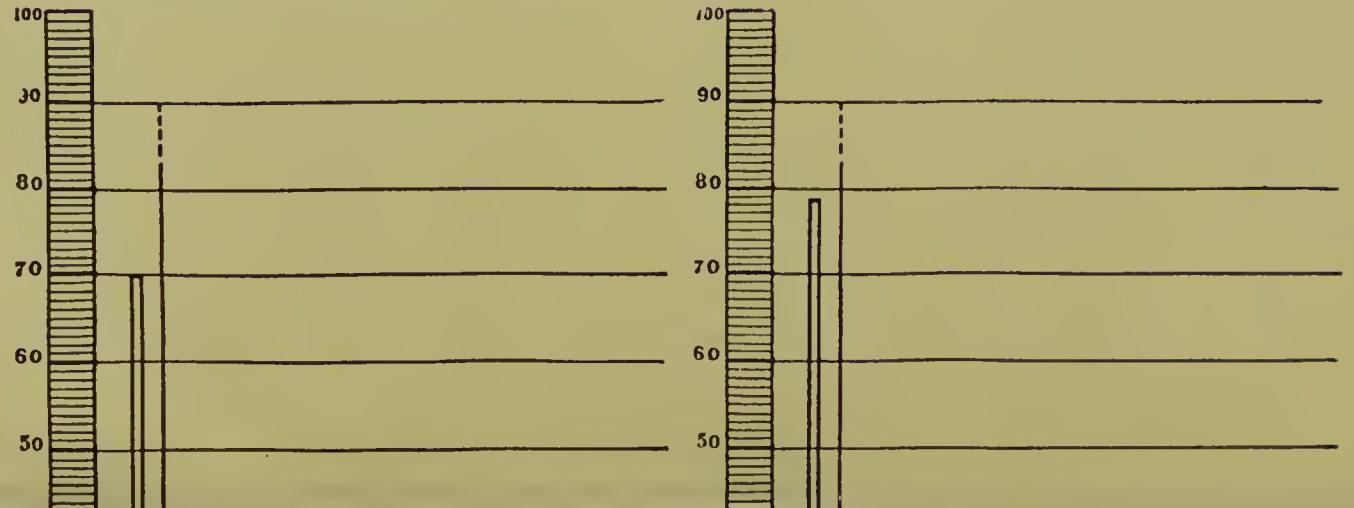
The case of dyspepsia is interesting: an auto-intoxication bas probably been going on here by absorption of organic acids and an excess of the aromatic bodies from the gastro-intestinal tract. The high ammonia percentage and the high ratio of the conjugated sulphates shows tbe strain that has been thrown on the liver in its efforts to neutralise the acids aud detoxicate tbe aromatic bodies. Tbe liver evidently temporarily failed and an urticarial attack is the result of the auto-intoxication. As soon as a purge and alkalies bad been given the urticaria disappeared. The disappearance of the urticaria is probably due to the flushing out of tbe seat of toxic production and a temporary reduction of the intestinal flora, and consequently diminution of the putrefactive processes engendered by them. The alkaline treatment, which is often found to be almost a specific in such cases, possibly acts by increasing the blood alkalinity which bas been lowered, or by reinforcing the body bases which have been attacked to neutralise over acid production.

The case of cirrbosis illustrates the increase in the ammonia ratio which I have referred to when speaking of the same case on Plate II. It will be remarked how the increase in the amount of the nitrogen of the ammonia diverted to neutralise the acid excess, and the increased amount of uric acid, has affected the columns $\frac{\text{nitrogen of urea}}{\text{total nitrogen}}$, which is 66 per cent. instead of from 83 to 90 per cent. The conjugated sulphate in this case was remarkably increased on the day this analysis was made, and it is interesting to note that the urobilin which was always high in this case increased to eight and ten times its normal amouut when increased intestinal putrefaction (instanced by the high ratio of conjugated sulphates) was present, being lowered to only twice or three times the normal when the conjugated ratio fell. It would appear that the highest function of the liver is to destroy (probably by oxidation) toxic products of all nature invading it from the gastro-intestinal tract. If owing to any cause tbis property becomes impaired it can still save the organism by neutralising and detoxicating these bodies by synthetic processes. This is evidenced by the power possessed by healthy livers of destroying urobilin excess, burning up organic acids and conjugating and neutralising that which they cannot destroy. A point to be noted in connection with this case of cirrhosis, is, that although the uric acid output was high there was no leucocytosis—the increase in the number of white blood cells is considered to be the cause of the increased uric acid output in leucocythemia.

PLATE V.

TWO ANALYSES OF THE URINE IN A CASE OF ENTERIC FEVER.

THE NORMAL RATIOS ARE ILLUSTRATED BY THE BLACK COLUMNS. THE DOTTED TERMINATIONS SHOW THE LIMITS WITHIN WHICH THE RATIOS MAY VARY AND YET REMAIN NORMAL. THE DOUBLE COLUMNS REPRESENT THE RATIOS ACTUALLY FOUND.



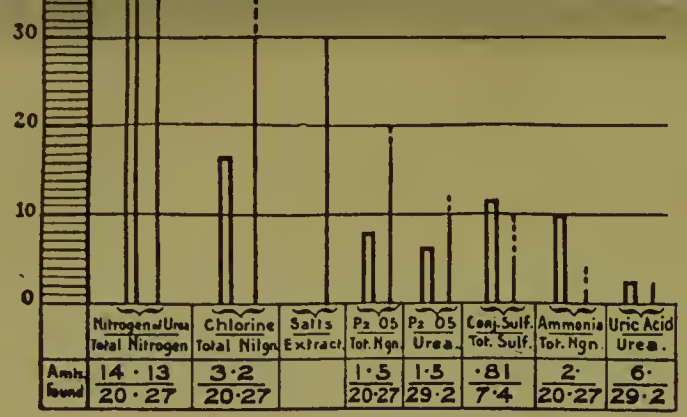


CHART 9.

Quantity ·74 litre.
Albumen present, traces.
Albumose present, traces.
Sugar—very slight traces.

Indican in excess.
Urobilin—excess.

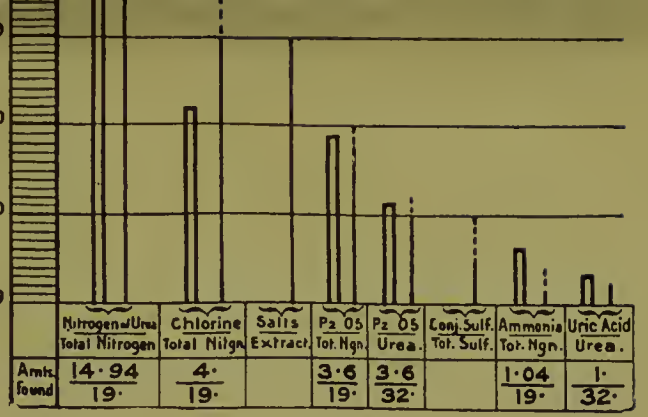


CHART 10.

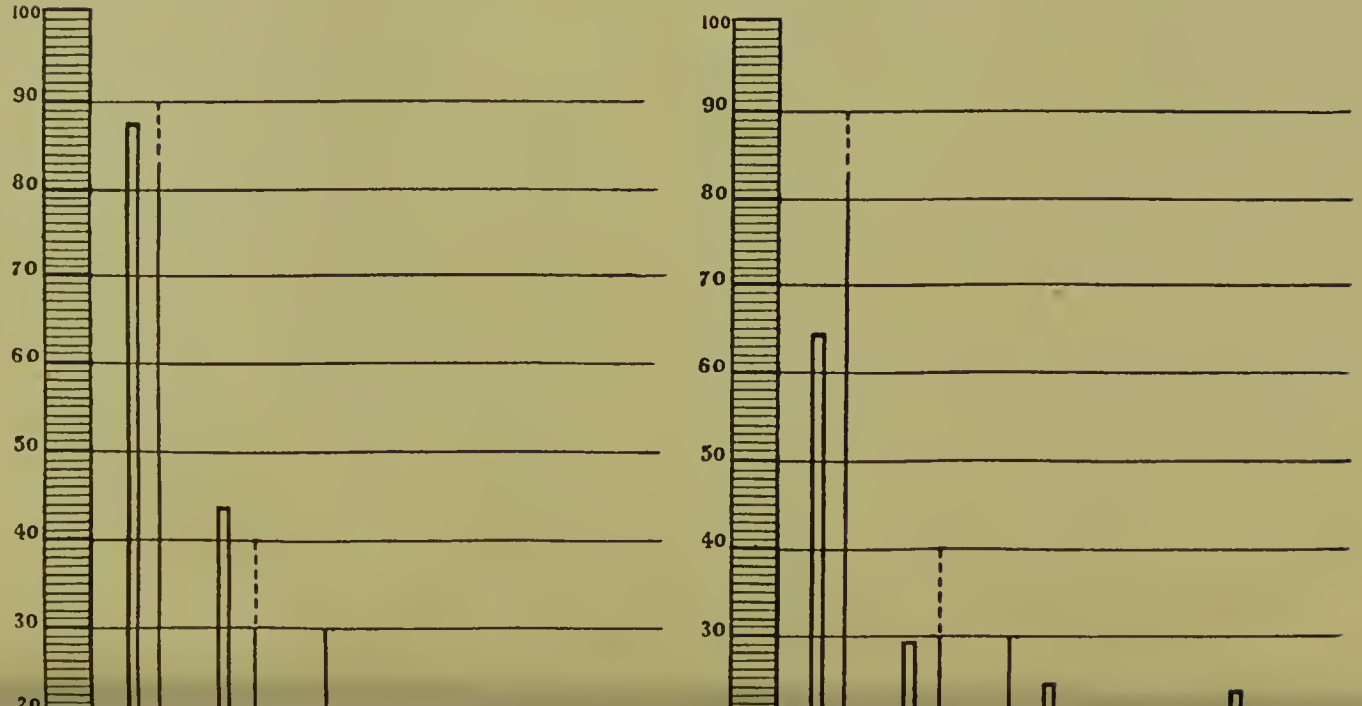
Quantity 1 litre. Albumen—faint traces. No Albumoses or Sugar. Indican not in excess.

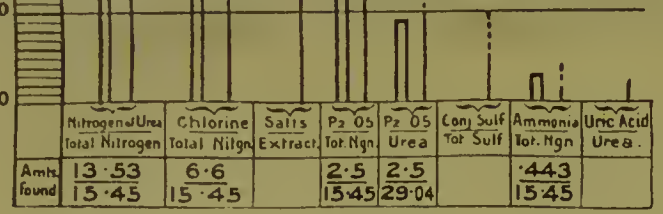
Diet milk and beef tea on both occasions.

M. A case of typical enteric fever from Dr. Dalton's wards in King's College Hospital. The first analysis was made at the end of the second week and the second at the end of the third. The case was not a severe one, the temperature fell to normal on the seventeenth day, the second analysis being made three or four days after it had become normal. The points to be noted are (1) the general improvement shown in the metabolism; (2) the rise in the ratio $\frac{\text{nitrogen of urea}}{\text{total nitrogen}}$; this ratio is a very important one in cases of fever, and especially enteric and other fevers of long duration. The column falls as a rule in proportion to the severity of the disease, and rises with convalescence. In certain cases of typhoid when there is rapid intoxication this fall is not always as marked as one would expect it to be, but in the ordinary run of cases this ratio varies directly with the severity of the case. In fact, Robin considered this ratio so important in cases of enteric that he divided his cases into severe and benign according to the height of this column. It will be seen that the ratio has risen from 69 per cent. to 79 per cent. in eight days. In this case, with the rise in the $\frac{\text{urea N.}}{\text{total N.}}$ column, there has taken place a fall in the ammonia column to normal limit. The rise in the columns for P_2O_5 and the chlorine column are indicative of the improved metabolism, there being no longer present the necessity for the blood to tenaciously guard its salts as occurs when the body is defending itself against serious disease.

PLATE VI.

CHART OF TWO CASES OF DIABETES.





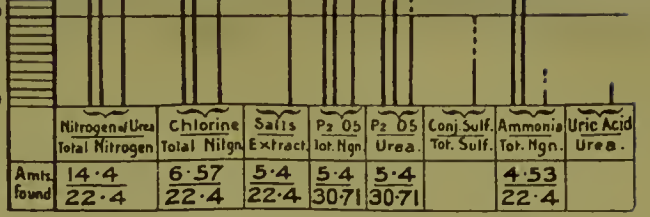
	Nitrogen of Urea / Total Nitrogen	Chlorine / Total Nitgn	Salts / Extract	P_2O_5 / Tot. Ngn	P_2O_5 / Urea	Conj Sulf / Tot Sulf	Ammonia / Tot. Ngn	Uric Acid / Urea
Amts.	13·53	6·6		2·5	2·5		·443	
found	15·45	15·45		15·45	29·04		15·45	

CHART. 11. CASE A.

5½ litres of urine passed in the 24 hours. Acetone and diacetic acid present. B. oxybutyric acid 270 grms. in the twenty-four hours. Sugar 8·6 per cent.

G. S. A case of diabetes in a man, aged 40; the disease has been present four or five years. During the last year the amounts of sugar and oxybutyric acid passed has been always considerable. The ammonia has risen to 20 odd per cent. of the total nitrogen when no alkalies have been taken, and the ammonia excreted has varied directly with the amount of oxybutyric acid in the absence of special alkaline treatment.

The patient is now on ordinary mixed hospital diet and is receiving 2 oz. of sodium carbonate a day by way of treatment.



	Nitrogen of Urea / Total Nitrogen	Chlorine / Total Nitgn	Salts / Extract	P_2O_5 / Tot. Ngn	P_2O_5 / Urea	Conj. Sulf. / Tot. Sulf.	Ammonia / Tot. Ngn.	Uric Acid / Urea
Amts.	14·4	6·57	5·4	5·4	5·4		4·53	
found	22·4	22·4	22·4	30·71	30·71		22·4	

CHART 12. CASE B.

5 litres of urine passed in twenty-four hours. Acetone and diacetic acid present. B. oxybutyric acid 179 grms. Sugar 4 per cent.

B. S. A case of diabetes in a young man, aged 21, who has had diabetes for two years, and three times been on the verge of coma, being relieved each time by the administration of large amounts of alkali. The blood alkalinity has been constantly subnormal, never above $\frac{N}{80}$ for some time past.

The patient is at present on a liberal diabetic diet and is receiving 5i. of citrat. of sodi. and xxx. gr. of bicarbonate every three hours.

REMARKS.

These two cases are interesting from several points of view. It will be noticed that while the output of B. oxybutyric acid in case A is greater than that of case B, the ammonia is much less, in fact, less than normal. This is entirely due to the enormous quantities of sodium carbonate being given. The alkaline base thus supplied is more than sufficient to neutralise the oxybutyric acid, and the result has been that the body bases (especially NH_3) have been spared. This man when not receiving these large amounts of soda excreted large quantities of ammonia, equal sometimes to 20 per cent. and more of his total nitrogen, and, further, the ammonia output varied directly with that of the B. oxybutyric acid.

In case B, although smaller amounts of B. oxybutyric acid are being passed than in A, the ammonia rises to 24 per cent. of the total nitrogen. Alkalies are, indeed, being given here also, but in less amount than in case A. (The sodium value of citrat. and bicarbonate of soda being given the patient: equal approximately ½ oz. soda carb. daily—*i.e.* a quarter of the amount being taken by case A.)

Since the analysis shown on Chart 12 was made the sodium salts being given to the case have been increased by about one-third to a half; the result has been that while the B. oxybutyric acid has been considerably *increased*, the ammonia percentage of the total nitrogen has fallen to 15 per cent. It often happens that the administration of alkaline bases increases the output of the B. oxybutyric; this is due rather to a sweeping out of the sodium oxybutyrate thus formed than to any increased formation of the acid.

By watching the amount of B. oxybutyric acid excreted and noting the ammonia ratio, one is enabled to see clearly the true condition of the patient and avert any impending coma—which it would be otherwise difficult to do. The value of the alkaline treatment was amply demonstrated in both cases.

The schema just described is the only one employed in cases of acute disease, though it may be filled in also to any required extent in nearly all chronic cases. The column schema being more reliable and accurate than the first or percentage schema, the deductions which may be made from it are more valuable.

As this paper is written with the object of drawing attention to the value of quantitative urinary analysis, I have considered it advisable to give four or five specimens of each kind of schema. It is impossible to attempt to describe or discuss the urinary curve in all pathological conditions without making the paper of undue length. I have therefore deemed it better to select and describe such cases merely as illustrate the value of the system and the object I have in view.

As the first four schemata illustrate the work of three separate observers who have not in all cases used identical analytical methods, some explanation on this point will be necessary.

The "normal per kilogramme" units of Gautrelet, *i.e.*, the amounts of urinary constituents which have been found experimentally to be excreted per kilogramme of healthy body weight, have been used in every case. These amounts are 24 c.c. of water, 1 gramme of extract, 0·5 gramme urea, 0·1 gramme chlorine, 0·05 gramme (P_2O_5), 0·03 gramme acidity if expressed as P_2O_5, otherwise 0·5 c.c. normal alkali solution, 0·001 gramme uric acid, and the same amount of urobilin per kilogramme.

The second factor necessary to find the individual normal has in all cases, except those analysed by myself, been the mean between the actual weight and theoretical weight for height. In the cases which I have analysed the actual body weight alone has been taken into consideration. The allowances made for age are those described in my earlier paper.

I am uncertain as to some of the methods used by Richard (one of the observers), but as there is little or no variation in the methods used by French chemists in urinary analytical work, one may assume that, with two exceptions to be described, the methods used by Gautrelet and Richard are similar to those used by myself and described in the last paper. The exceptions mentioned are in the method used for estimating (1) the extract of total solids, and (2) the urea. In France the extract is generally estimated by evaporation and then drying at 100° C. I have estimated the solids by densimetry, the specific gravity having been previously determined with great care by weighing.

As far as I am aware, neither Richard nor Gautrelet eliminate the preformed ammonia or kreatinin before doing their urea estimations; consequently the results in their cases will be to some degree erroneously higher than those obtained by the process I use. The fact that the processes used have not in all cases been identical seems, in my opinion, to enhance rather than diminish the value of the results, showing as it does that alterations produced by disease on the nutritional rhythm quite overshadow the smaller differences due to different analytical methods.

Gautrelet's analyses are taken from his most recent work ("Spectroscopic Critique des pigment urinaires normaux"), while I am indebted to Dr. Gilchrist, of Nice, for his kindness in allowing me to publish some of his cases analysed by Richard (Analytical Chemist of Nice).

The column schemas illustrated on plates 4, 5 and 6 have been filled in by myself throughout, and the methods used have been those already described by me in my first paper. In two cases the urinary acidity has been determined by the new process of Folin, the organic and mineral acidities being determined separately, and the total acidity estimated by phenol-phthaleine in the presence of oxalate of potassium.

The schemas which have been given illustrate, I venture to think, that a quantitative urinary analysis has its uses. Charts 7 and 8 are examples of the fact that a qualitative analysis alone is not always sufficient, as a urine may show no abnormality to such tests and yet remain gravely abnormal. The tracings of cirrhosis, gout, tubercle, and dyspepsia show that particular diseases appear to affect the metabolic exchange, each in a special manner. A quantitative analysis may thus be helpful in aiding or confirming a diagnosis. The column schemata of enteric and diabetes are illustrative of the value of a quantitative analysis in the prognosis of disease. The condition of the metabolism is well portrayed in a column schema, and it is evident that the capacity for resisting disease and the chances of recovery depend on good nutrition. In the case of a disease like diabetes a quantitative analysis is absolutely essential; it shows us the exact condition of the patient, whether he is or is not in danger, and the lines on which we should treat the case. To further illustrate the value of quantitative urinary work, I propose to take up in turn each of the urinary constituents generally analysed, and point out the knowledge which

may be gained for diagnosis and prognosis by variations in the amount of their output or ratio. Though objection may be taken to such a method of treating the subject as more suitable for a book than an article such as this, I have adopted it on account of its simplicity and conciseness.

Volume.—Although so easily done, it is not often that one sees the urinary volume carefully measured, and variation in its amount laid stress on ; yet much information may be derived from a study of the quantity of urine passed. I do not, of course, refer to variations in the urinary volume of a temporary nature and due to well-known causes, such as climatic influence, or the amount of fluid taken, but to that persistent deviation from the normal seen in some pathological conditions. I will not dwell on the polyurias of diabetes and the small red kidney, or on the increased volume which heralds early kidney irritation, whether due to cancer or tubercle. These conditions are as well recognised as the oligury accompanying acute diseases generally, and especially acute kidney disease and the specific fevers, and require no comment. I would, however, like to call attention to a few conditions not so well known or understood. One of these is the polyuria often seen in the earliest stages of tubercle and sometimes accompanied by an increased output of mineral matter. This polyuria and demineralisation which Robin has fully described in his " Etudes cliniques sur la nutrition dans la phthisie pulmonaire," he ascribes to the vital reaction of the organism to the tubercular attack. Whatever the reason may be, quantitative analysis might be of use in such cases of early disease and would perhaps help the diagnosis in obscure cases. It has been stated by Gerard that, while the diurnal urinary output of the healthy is in excess of the nocturnal, the reverse of this holds good in disease. The amount of the urinary volume is of the greatest importance in cases of acute and grave disease. Here, owing to loss of water through other channels (sweat, diarrhœa, &c.) or a diminished intake, there is a severe drain on the body water which is so necessary to nutritive exchange and perfect metabolism. The condition so produced Herter considers may partially account for the torpor seen in severe diseases such as enteric fever, the immediate cause being the anhydrous condition of the central nervous system. When one is analysing the urine of diseases such as enetric and notices the highly concentrated fluid full of imper-

fectly elaborated end products, many of which are abnormal and toxic, one feels that cases such as these would do better if the body were more thoroughly flushed out, diuresis encouraged by the ingestion of much larger quantities of water than is usually given to acute cases, and a natural diuretic like common salt given more freely.

In acute kidney disease the volume as well as the solids passed in the urine is, of course, one of the most important points in the prognosis of the case. This is a well-known fact, but it does not always receive the attention it deserves.

Finally, a knowledge of the urinary volume in acute kidney disease, or in such cases as mitral heart affections, may help us to take early to diuretic measures and so avert an impending uræmia, or improve the condition of a water-logged body.

The Extract.—The amount of the urinary solids can be very rapidly estimated with sufficient accuracy for clinical purposes by a Westphaal balance, and densimetry, and they are worth estimating, as useful information may be acquired from a knowledge of their amount. In acute disease any great increase may indicate the severity of the condition, being a measure of the katabolic intensity. If exudations are to be removed, a knowledge of the amount of solids passed will show us what Nature herself is doing to solve the problem, while a deficiency of solids may point to defective elimination, especially when there is fever present and increased katabolism (Purdy). Of course the amount of solids present must be calculated from the urine of the twenty-four hours. Individual samples vary markedly in their density. The ratio $\frac{\text{salts}}{\text{extract}}$, which is given in the column schemas, is a useful guide in some conditions, such as the early tuberculous condition already mentioned.

The Urinary Acidity.—Round the question of the acidity of the urine a great deal of argument and speculation has centred, and yet the problem seems no nearer solution. That the urine is acid—that is, the cyclical urine, for the urine passed after a meal of vegetables or fruit may be alkaline—is evident. It is no less evident that in certain pathological conditions, such as the gout, cirrhosis, and dyspepsia schemas illustrate here, and in many others, the acidity is greatly increased as it may be greatly diminished in other pathological states.

The urinary acidity has been considered so important by some

that they have grouped diseases under two main divisions—diseases of the hyper-acid diathesis and those of the hypo-acid ; the divisions being dependent on the reaction of the urine (Gautrelet, Bouchard, Joulie, and others). While one cannot subscribe to statements so sweeping as these, the fact remains that in certain conditions, such as gout, acute rheumatism, diabetes, cirrhosis and others, the urinary aeidity is generally markedly and constantly increased, while the opposite holds good as a rule in tuberculous and scrofular affections.

The estimation of urinary acidity is thus an important matter, but is unfortunately beset with many difficulties. The urine is a mixture of bases and aeids, the aeid ions predominating in normal urine. This mixture gives very different results, according to the indicator used. Phenol-phthaleine gives higher results than litmus, while litmus gives an amphoteric end reaction and phenol-phthaleine an uncertain one owing to the presence of ammonium salts. The difficulties may, however, be surmounted if any one method be used for all cases ; such as if neutral litmus paper be always used, or Folin's method with phenol-phthaleine (using an oxalate to destroy vitiating influence of the ammonium salts). The neutral litmus method has been used in all the percentage graphics, while the more reeent method of Folin has been used by me in all later work, and is the one employed in the eolumn schemas shown here.

In such a mixture of acids and bases as the urine contains it is difficult to assign the urinary acidity to any one particular aeid salt. Up till quite recently, however, it was generally aecepted that the acidity was almost entirely due to the acid phosphate of soda. Folin, however, in a recent contribution to the *American Journal of Physiology*, appears to prove that organic aeids play a mueh more important *rôle* in determining urinary acidity than has hitherto been aseribed to them. He shows, in faet, that the acidity of many pathological urines is ehiefly due to organic aeids. In the sehemata illustrating the cases of eirrhosis and urticaria it will be seen that the organic aeidity worked out on Folin's method is greater than the mineral acidity. This is also often true of the acidity of diabetic urine. The amount of ammonia present in these cases is to some extent an index of the organic acidity as it is the base used by organic acids.

The amount of urinary acidity is of value as showing the amount

of acid being produced in the body and thrown into the blood. The reaction of the blood in health, as is well known, is of constant and unvarying alkalinity (N/35 Wright's method). This state of blood alkalinity is so vital to our health that the condition is maintained at all costs. The renal epithelium is one of the agents at work to maintain constancy in blood alkalinity. The process is probably a vital and selective one, the excess of acid ions being got rid of as quickly as they appear. Thus in health an increased urinary acidity would not mean a diminished blood alkalinity, but would indicate that a disproportionate amount of acid ions were being produced—thrown into the blood and instantly thrown out again. In certain pathological conditions, however, the blood alkalinity is diminished, often markedly. Perhaps in these cases the vital activity of the renal epithelium is impaired, or is unable to deal efficiently with the greatly increased acid production.

The acidity of the urine can never be considered so important as the blood reaction, as it does not always reflect the condition of the blood probably even in some pathological conditions. As, however, urinary acidity is so readily estimated, and is evidence of body acid production, its amount should always be noted.

The total acidity of the urine, as shown by neutral litmus, works out to about 0·5 c.c. normal alkali per kilogramme of body weight. The increase and decrease of acidity are well shown by the percentage schemata.

One or two points in connection with urinary acidity may be mentioned. Nitrogenous diet quickly increases it owing to the liberation of sulphuric and phosphoric acids. Cheese has the same effect owing to its poverty in alkaline bases and richness in casein (a nucleo-albumen and precursor of uric acid). For the above reasons cheese should be forbidden the gouty, and the nitrogenous part of their diet reduced.

In connection with gout a tradition which will die hard is the supposed efficacy of the salts of lithia in this disease. The reason given being that the urate of lithia is the most soluble of all urate combinations. The utter worthlessness of the treatment is well pointed out by Bunge, and will be realised when it is remembered that by Berthellet's law acids distribute themselves to the bases present directly in proportion to the amounts of these bases. The amount of lithia absorbed being but a few grains, the amount of uric acid apportioned to it would be infinitesimal.

As the acid phosphate of soda plays so important a *rôle* in urinary acidity, it was only to be expected that it would be an efficient acidifier of the urine. This has been demonstrated by Robert Hutchison in a recent contribution to the *British Medical Journal*. If given in doses of from 30 to 40 grains it will be found a useful drug in conjunction with urotropin when it is desired to keep the bladder acid and aseptic after operations, or in order to inhibit bacterial action.

The Chlorides.—In both the percentage and column schemas a place is found for the urinary chlorine. It might be thought that a product like chlorine, the excretion of which is so easily influenced by the food taken, could have no clinical significance. This is not so; for while variation in the chlorine excretion in health has little significance, this does not hold good in acute disease. The blood, which so carefully guards its alkalinity, guards its molecular concentration with equal care. If from any cause this concentration has been altered or threatened by losses of salts (chiefly chlorides), the blood retains its salts until the molecular concentration is restored. Common causes of salt retention are the pulmonary and pleuritic exudations seen in pneumonia and acute pleurisy, and chlorine is retained after all hæmorrhages, whether accidental or produced by operation. A low chlorine output may, however, be seen in cases of severe disease, the cause of which cannot always be traced to an increased loss due to any of the causes just mentioned, or to a diminished intake.* In such cases a marked fall in the chlorine output is a grave sign, for it tells us that the blood is actively defending its molecular richness. No particular prognostic value can be assigned to an increase of the body chlorides in disease, but generally it holds good that, apart from the influence of food, such an increase coincides with improvement and convalescence. A point of practical interest is the diuretic action of salt. It appears (as Bunge says) as if the products of metabolism cannot be

*N.B.—It is difficult to account for the markedly diminished chlorine excretion in febrile conditions. While the chlorine in fever is diminished, irrespective of diet and even of increased chloride consumption, there is no increase of the chlorine tenure of the blood or tissues in fever, and chlorine is not got rid of in increased amount by any known excretion. A very complete account of chlorine retention in fever is given in a paper which Dr. Garrat has published, since these papers were written, entitled "Metabolism in the Febrile State of Man."

eliminated in aqueous solutions alone, but require a saline solution for the purpose. For this reason salt is most useful in uræmia, as it stimulates renal excretion, especially when injected *per rectum* or infused. Both on account of its diuretic action and its value to the blood, it would, I think, be advantageous to increase the amount of salt given in acute disease. If salt were given, it would help to maintain blood concentration, and at the same time, by promoting diuresis, flush out the imperfectly elaborated products caused by the impaired metabolism associated with acute disease.

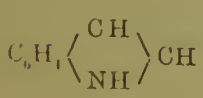
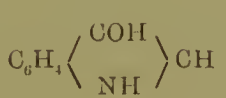
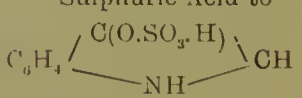
A point of interest, but not of much clinical value, is the effect of vegetable foods on the excretion of chlorine. The potassium, which is so plentiful in vegetable foods, takes up the blood chlorine with avidity when it is absorbed, and as the blood does not tolerate the presence in excess of any one salt, the potassium chloride is eliminated, thus increasing the chlorine output. Vegetable feeders require plenty of salt to compensate for the chlorine loss caused by the high potassium tenure of their food, and it is for this reason that salt is so essential to the rice-eaters of Japan and Bengal, while his language has no name for salt with the meat-eating Esquimaux. Although generally speaking a chloride excess means little, there are two conditions in which one often sees a persistent excess in the chlorine output. These conditions are tubercular disease and certain dyspepsias (see charts 4 and 6). It is difficult to assign any reason for the increase in the cases of dyspepsia which are generally of the type known as acid, with diminished hydrochloric acid and excessive fermentation—unless it is the diminished elimination of chlorine by the gastric membrane, or the abnormal appetite occasionally present. The chlorine increase so often associated with tubercular disease, Robin assigns—as I have already stated—to the demineralisation of the tissues which appears to herald the tubercular attack.

The Phosphates.—What has been said of chlorine retention might equally be applied to the retention of phosphates in acute disease. In such cases the ratio column $\frac{P_2O_5}{\text{Total } N}$ is invariably low, the reason being, as with chlorine, the necessity of up-keep of blood concentration. In acute kidney disease the phosphate excretion is strikingly low. Purdy considers phosphate diminution to be almost as infallible a sign of such conditions as the presence of albumen. An increase of the phosphate ratio is seen

in true phosphaturia—which is, however, not a common affection, most of the so-called phosphaturias being nothing more than a deposit of earthy phosphates due to the abnormal alkalinity of the urine. In nervous disorders, and especially during nerve crises, the phosphate ratio is often increased. Phillips and Schaffer, working on a case of alternating insanity, have shown how markedly the phosphates may rise on the " mad " days.

The Sulphates and ratio $\frac{\text{Conjugated Sulphates}}{\text{Total Sulphates}}$.—As the sulphate output in the urine is derived almost entirely from body metabolism and the total sulphates vary directly with the output of nitrogen, no inference of clinical value can be drawn from a knowledge of their amount. Variations in the ratio $\frac{\text{Conjugated Sulphates}}{\text{Total Sulphates}}$ are, however, as important as they are interesting to study. In health nine-tenths or more of the sulphuric acid is eliminated in combination with alkaline bases such as sodium and potassium, the remainder is found combined with certain aromatic radicles. The aromatic bodies found in conjunction with this portion of the sulphuric acid are indol, skatol, kresol, and phenol. These hydroxyl substitution products of benzene, as Hopkins describes them, appear to be derived from the by-products of trypic digestion. While phenol $C_6H_5OSO_3OH$, and kresol C_6H_4 (CH_3) OSO_3 are directly sulphated, the indol and skatol become sulphates in a more complex manner, but one so interesting as an illustration of a protective body synthesis, that I shall briefly describe it.

Indol and skatol are too toxic to be absorbed into the circulation unchanged, so the liver proceeds by first oxidising and then uniting them to sulphuric acid, to detoxicate these bodies. The process is as follows: The indol and skatol taken up from the intestine are carried by the portal vein to the liver and there, oxidised to indoxyl and skatoxyl, they are then held in loose combination with the protoplasm of the liver cells till sufficient SO_3 has been collected to sulphate them (Herter).

Indol.	Oxydised in liver to Indoxyl;	And afterwards coujugated with Sulphuric Acid to
		
		(Indoxyl. Sulphuric Acid.)

The liver acts as a living screen protecting the blood and especially the central nervous system from these toxic bodies. It seems reasonable to suppose that in cases of intestinal putrefaction the constant call on the liver to detoxicate the excess of aromatic toxic bodies produced, may, by impairing its efficiency, cause a leakage of these products into the circulation. One sees cases often enough clinically of headache and neurasthenia associated with chronic constipation. Another point that strikes one is, that the strain thrown on the liver by excess of such work may permanently affect its efficiency if the cause is continued long enough, rendering that most vital of all organs a less efficient bacteria and toxin filter. It is quite possible that a predisposition to such diseases as enteric, dysentery, or cholera, may be so induced.

The main cause of an increase in the conjugated sulphates, is intestinal putrefaction, the direct cause—as Herter has experimentally shown—being the colon group of bacteria. In all conditions where intestinal putrefaction is increased (obstruction, constipation, or deficiency in the gastric and intestinal secretions) the conjugated sulphates are increased. The high conjugated ratio seen sometimes in diabetes, neurasthenia and some anæmias, is probably due to intestinal causes. Putrefaction elsewhere, such as is caused by long-continued suppuration in the tissues or phthisical lung cavities, also causes an increased excretion of conjugated sulphates owing to the formation of aromatic bodies, similar to those produced in the intestine in putrefaction. In the charts which are given, it will be noticed that the conjugated sulphates are estimated conjointly, any other course would be clinically impracticable. The most important of the group, however (indol), is separately searched for ; its presence is so easily determined that this should always be done. In young children indol should be present in but faint traces ; any appreciable reaction shows the condition of the intestinal tract to be pathological. In adults the amount of the conjugated sulphates present will be indicated to some extent by the character of the indican reaction. Some of the abnormal colours occasionally seen in urine are due to the aromatic bodies just mentioned, or to closely allied bodies. Indoxyl and skatoxyl are chromogeus and may, as such, be oxidised to indoxyl blue (indigo) or skatoxyl red respectively. When these bodies are combined with sulphuric acid, oxidation cannot

take place, and so ordinary urine does not become blue or red on exposure to air. In some pathological conditions, however, owing possibly to putrefaction in the urinary tract itself, the indoxyl sulphate gets broken up and its liberated indoxyl is now oxidised to indigo. The brown colour of the urine in alkaptonuria is due to the aromatic carboxy acids—homogentisic acid and uroleucic acid. The much more familiar green-brown colour of the urine seen after the excessive absorption of carbolic acid, is due to oxidised products of this aromatic body—hydrochinon and pyrocatechin.

Urea and the ratio $\frac{\text{Urea Nitrogen}}{\text{Total Nitrogen}}$ find a place in both kinds of schemata. The output of the urea, and especially variations in the ratio just given, are of distinct diagnostic and prognostic value. Urea is the simplest of all the nitrogenous end bodies and may be looked upon as the physiological end product of nearly all the nitrogenous matter consumed. This simple body with a molecular weight of only 60, is produced in such large amount that its birthplace and the processes by which it is produced are probably many. Urea may be likened to the last link of a chain, the first link of which is the huge and complex albumen molecule, containing its thousands of atoms. This unwieldy albumen molecule is gradually broken down—probably by a process of hydrolysis, through at first complex and then more simple bodies—amides, then leucomaines, and finally ureides to urea. While much of the urea comes from the ureides, some also comes from combinations and changes high up in the chain (union of glycocol with the amides), &c. Most of the urea, however, owes its formation to a splitting off of NH_3 from the albumen molecule itself and the junction of this ammonia with CO_2 in the liver. This origin of urea is the most important and interesting clinically. While the albumen molecules are being absorbed through the intestinal wall, ammonia is, by some unknown agency, split off from it and carried in large quantities to the liver, when it unites with CO_2 of the blood and tissues to form urea, $CO<^{NH_2}_{NH_2}$, an amide of carbonic acid. There is no doubt whatever about this origin of urea, for the portal vein is full of ammonia, while the hepatic vein contains practically none; and if the liver be experimentally shut off from the circulation (Eck fistula in animals) the urea is very greatly diminished and the amount of ammonia correspondingly increased.

Ammonia itself is toxic and the synthesis of NH_3 and CO_2 is

possibly a detoxicating synthesis allied to the synthesis described in the formation of the conjugated sulphates, urea being the detoxicated product.

In health then, the NH_3 and the CO_2 join to form urea. The ammonia may, however, be diverted from this, its natural, task, if a more urgent one presents itself. This happens when an acid or acids have been produced in larger quantity by the body than the liver can oxidise or destroy; ammonia being a base, and a plentiful base at that, unites with the acid which, like the ammonia, is harmful alone, the result is an innocuous and neutral salt.

As the conditions favourable to urea production are (*a*) an efficient liver, and (*b*) little or no acid absorption from the intestinal tract, it is no surprise to find that in severe liver disease such as cirrhosis, acute atrophy, cancer and abscess, and in all cases of excessive acid absorption, the urea output is diminished, either actually or relatively, while the ammonia is increased.

While the lowness of the column $\frac{\text{Urea N}}{\text{Total N}}$ is often due to the increased ammonia alone (chart 12), in such cases, it may also be caused by an increase of the nitrogen of the uric acid and the extractives generally (chart 8). It is considered by some that uric acid is a precursor of urea, as I will show later on. In convalescence from acute liver affections, the urea ratio rises and the actual amount is also increased. In point of fact in catarrhal jaundice convalescence is said to be often ushered in by a sudden increase of the urea and urinary volume. While about one-half of the urea is produced in the liver, the other parts of the body produce the rest. In acute and grave diseases of whatever nature, when the metabolism has been much affected, the urea nitrogen column falls (charts 9 and 11), the loss being accounted for by the increase of the incomplete end products called extractives. So important have some authorities (Robin, Grubel) considered the $\frac{\text{Urea N}}{\text{Total N}}$ ratio that they have divided their cases (chiefly enteric) into benign and grave according to the height of the column.

In the charts of enteric fever (charts 9 and 10), cirrhosis (chart 8 and one case of diabetes (chart 12) it will be seen that the $\frac{\text{Urea N}}{\text{Total N}}$ ratio columns are much lower than normal. This condition is due to an increase in the uric acid nitrogen (cirrhosis), extractive nitrogen (enteric), or that of ammonia (diabetes chart).

The amount of urea excreted in acute kidney disease is important, as it enables one to estimate the functional power of

the kidneys and be warned of an impending attack of uræmia when the constantly decreasing output shows the waning efficiency of the renal filter. The output of urea is actually though not relatively increased in all conditions where tissue metamorphosis is also increased (such as fever), or when an excess of meat food is being taken as in diabetes. The excretion of urea is often increased in phthisis, owing probably to increased katabolism.

Preformed Ammonia and its ratio $\frac{NH_3}{\text{Total N}}$.—When writing about urea, I mentioned that one of its chief sources was the union of NH_3 and CO_2 in the liver. I further mentioned that in cases when there was danger of an acid intoxication, the ammonia was diverted from its usual course and was given up to neutralise such acids. Ammonia is the most abundant and least vital of the body bases, other and far more valuable ones being the sodium and potassium of the blood. Whenever there is an increase in the amount of body acid production or interference due to disease with its normal acid oxidising and destroying functions, the urinary ammonia rises.

Increased ammonia production may be due to (1) excessive meat food (here acids are in excess of bases) and to the katabolism which it stimulates, the body katabolism itself increasing acid production. (2) An excess of organic acids is produced in the gastro-intestinal tract in dyspepsia and gastro-intestinal fermentations, and failing to be burnt up owing to the concomitant inefficient state of the liver, take up ammonia as a base and increase its excretion (chart 7). (3) In certain specific fevers, in arthritis deformans, but most important of all in diabetes, when the B. oxybutyric acid may amount to over 200 grammes in the twenty-four hours (charts 11 and 12). (4) In all conditions of liver insufficiency due to whatever cause, cirrhosis, cancer, acute yellow atrophy, &c., the increased output is here due to two causes (*a*) the inability of the liver to carry out its proper function of urea formation; (*b*) the diminution of its power of acid oxidation (chart 8).

In some of the above conditions the blood alkalinity may be reduced, especially so in diabetes, in fact in diabetic coma the blood may become acid through over-production of acids and the inability of the body to neutralise such large amounts properly.

In connection with ammonia excretion, I trust a word or two about B. oxybutyric acid may not be out of place. This acid, which is closely allied to the better known bodies—acetone and diacetic

acid—is found in most cases of diabetes and to a small extent in some other conditions (specific fevers, &c.).

The amount of this acid excreted is more important than the amount of the sugar, for it is the cause of diabetic coma.

The two cases of diabetes given illustrate very well how necessary it is to know the amount of acid present. In case B. (chart 12), the amount of ammonia being excreted is 23 per cent. of the total nitrogen, and the oxybutyric acid 169 grammes or 2·89 per cent.* Here it is evident that the body bases are being used up. In the first case A. (chart 11), while the oxybutyric acid reaches the enormous amount of 4·9 per cent., the ammonia is actually below normal. The fact is due to the large amount (2 oz. daily) of sodium carbonate being taken by the patient, which by neutralising the acid saves the body base ($N.H._3$). The amount of ammonia passed is a rough index of the quantity of B. oxybutyric acid present, but is not absolutely reliable, as occasionally the other body bases such as the sodium and potassium of the blood are early attacked. As a rule, however, the cheapest body base ($N.H._3$) is first yielded up, and when it is necessary to yield up the more valuable bases, the condition must be pronounced grave and the patient is within the danger zone of coma. The coma of diabetes is considered to be due to the fact that when very large amounts of B. oxybutyric acid are produced, the first body defence—the ammonia—is overcome and the more vital sodium and potassium bases of the blood attacked. The acid by combining with them prevents their carrying away the $C.O._2$, produced in the tissues to the lungs in the form of carbonates, as they usually do. The consequence is that CO_2 collects in the tissues and causes coma. Though this theory has lately been attacked (Beddard, Pembrey and Spriggs) and may some time be disproved, the fact that B. oxybutyric acid is the cause of the coma seems undoubted.

Uric Acid and the ratio $\frac{\text{Uric Acid}}{\text{Urea}}$ will be found mentioned in both schemata, the actual quantity in the percentage schema and the ratio in the schema of columns. I have retained this ratio in the schemata because although the variations of uric acid have not the diagnostic value at one time ascribed to them, yet as the uric

* N.B.—Since this paper was written, in September, 1903, I have satisfied myself that the amounts of oxybutyric acid here given, and estimated by the polariscope, are too high. The relative value of the comparison is, however, not affected.

acid is found to be abnormally high in certain conditions, it is well to have some system of recording the fact. The amount of uric acid produced was at one time considered to vary directly with the thoroughness of oxidation in the blood and tissues, and was considered to be a half-way product of urea. It is now generally recognised that the chief source of uric acid and of the allied purin bodies is the katabolism of nucleins, whether derived from the food or the tissues. Richet has, however, separated out a ferment from the liver which he calls a uroproteic ferment, and which he states is capable of transforming many nitrogenous bodies, including uric acid, into urea. It is probable that some of the urea may be formed in this way. In the charts which have been given earlier in the paper, it will be observed that the uric acid ratio column is particularly high in one of them, viz., the case of cirrhosis (chart 8). The twenty-four hours' output of uric acid is also seen to be high in chart 3 in both the tracings of cirrhosis cases.

As a matter of fact in cases of hypertrophic cirrhosis, as in many other liver diseases (acute yellow atrophy, cancer, &c.), the uric acid output is nearly always increased. This increase may be due to the increased destruction of liver cell nuclei, which takes place in these conditions (Herter), or, as Richet suggests, to the diseased condition interfering with the function of the uroproteic ferment. In stationary stages of gout, as will be seen by reference to tracings A. and B. in schema 5, the uric acid output is below normal; this is probably due to defective elimination, for, as will be seen on the same schema (tracing C.), the output is greatly increased towards the end of an acute attack.

The important part played by body nucleins in uric acid formation is well shown in cases of leucocythemia, when the uric acid output is greatly increased, owing to the nucleins derived from the disintegrations of these cells.

The amount of uric acid crystals found in urinary deposits, or the urate sediments so often seen, are, of course, no index of the amount of uric acid present. Uric acid is eliminated in the form of quadurates of soda; these are hyperacid unstable salts which rapidly break up into biurates and free uric acid. The biurates thus formed are acted upon by the acid phosphate of soda present in the urine, and are again changed to quadurates, which again in their turn are broken up to biurates and free uric acid. This reaction goes on till all the uric acid may be set free

(Sir W. Roberts). The activity of this exchange depends upon the urinary acidity, amount of acid phosphate present and the concentration of the urine. Uric acid calculi in the kidney are probably due to excessive urinary acidity causing a deposit of uric acid in the organ, for reasons just described.

The Urobilin.—Of the many urinary pigments urobilin is the only one which is regularly estimated. This is because of its characteristic and easily-recognised spectrum and its clinical significance. Urobilin is derived, as are all the urinary pigments, from the hæmoglobin of the blood ; but for the most part its immediate precursor is the bilirubin of the bile. The origin of urobilin is now recognised to be three-fold : (1) Directly from blood hæmoglobin by its reduction in the body ; (2) from bilirubin by reduction due to bacteria in the intestine ; (3) from both bilirubin and hæmoglobin in the liver. It will be seen by this that any condition which causes hæmolysis, should increase the output of urobilin, and this actually occurs in specific fevers and pernicious anæmia. For similar reasons there is an increased amount in the urine of those suffering from internal hæmorrhages and blood extravasations. There is great dispute as to whether the hepatic or the intestinal origin of urobilin is the more important. Clinically the urobilin of the urine is increased in most hepatic disorders ; including jaundice. This point seems to favour the hepatic theory of origin, but many argue that the urobilin is really almost entirely formed in the intestine, and it is because the liver is diseased and cannot destroy the excess that the urinary amount is increased. The uncertainty about its main source of origin does not militate against the value of urobilinuria clinically. It is a sign of hepatic disorder, excessive intestinal putrefaction, or hæmolysis.

The case of cirrhosis given in this paper illustrates well the probably manifold origin of urobilin. While this pigment was always high in this case (chart 3), it was further and greatly increased on one occasion when the presence of increased intestinal putrefaction was evidenced by the high ratio of the conjugated sulphates (chart 8).

All the urinary constituents quantitatively estimated in the schemas given have now been mentioned. No reference has been made, however, to urinary qualitative analysis, or the clinical significance of albumen, sugar, the bile pigments, &c., in the urine, as these papers were intended to deal with the less known side of urinary work.

This paper has already reached limits which were never originally intended, or wished for, but before concluding, I would like to call attention to the importance of testing for the presence of albumoses and peptones* in the urine. These are generally due, when present, to their leakage through a damaged intestinal wall, or their absorption into the blood from internal suppuration or necrosis. The presence of albumoses and peptones may tell us the amount of damage which has been done to the intestinal wall by disease, or help us to diagnose obscure cases with deep-seated suppuration. The close association between the presence of oxalic acid in the urine and excessive gastro-intestinal fermentation has been so clearly shown by Helen Baldwin's experiments on dogs and quoted by Herter (producing oxalaria by inducing a gastritis due to excessive sugar consumption), that it should be searched for in gastric disorders.

The schema of columns which I have put together is but a tentative arrangement and liable to alteration. Two new ratios which may soon be added or displace others in it are: (1) The ratio of $\frac{\text{Organic acidity}}{\text{Total acidity}}$ (Folin's method); and (2) the ratio $\frac{\text{Carbon of urea}}{\text{Total carbon}}$. The former ratio cannot as yet be adopted, as I am quite uncertain as to what the normal proportion is—without this guide the ratio cannot be graphically recorded. The second ratio, $\frac{\text{Carbon of urea}}{\text{Total carbon}}$, I am convinced is of great importance. While the non-calculated† nitrogen of the urine, *i.e.*, nitrogen of the extractives, forms but 6 to 7 per cent. of the total amount in the urine, and does not vary considerably, the unknown carbon is in greater amount and in pathological conditions varies considerably. Donzé and Lambling have shown that the non-calculated carbon comes chiefly from bodies like the pentoses, pentosanes, animal gum and oxyproteic acid. Work has been commenced on this ratio, but the results are at present far too few to justify its being used in the schemata.

In concluding, I would like to thank Drs. Dalton (King's College Hospital), Cayley (St. Mary's), and Beddard (Guy's), for permission to examine the urine of their patients and to make use of the charts drawn from some of these analyses.

To Dr. Gilchrist, of Nice, I am greatly indebted both for those

* Many deny that peptones are ever found in urine.

† By non-calculated is meant the nitrogen and carbon other than that accounted for in urinary constituents usually estimated (urea, uric acid, ammonia, &c.).

of his schemata which he has allowed me to publish and the constant encouragement and advice given me in my endeavours to work at urine. Dr. Gilchrist's collection of percentage schemata (Gautrelet's type) are probably unique and represent the work of years. Colonel Firth has given me great encouragement in my work and help in the production of these papers, and I take the opportunity of thanking him for it.

A short bibliography supplementary to that given in the first paper is added for the reference of those interested.

CLINICAL URINARY ANALYSIS.

A Critical Study.—Paper III.

(*Reprinted from the "R.A.M.C. Journal" of March*, 1904.)

As it has been suggested to me that fuller notes on the detail of methods employed in quantitative work might be useful to any interested in the subject, or likely to take it up, I have put together from my notebooks the methods which have been found the most practical and simple, yet of sufficient accuracy for all clinical purposes. The notes have been gleaned from many sources, and considerable time and trouble has been expended in the selection of methods.

Until quite recent years quantitative urinary analysis has been too complicated and laborious to be undertaken by any but the analytical chemist or those engaged in researches on chemical physiology and pathology. Now, however—chiefly abroad—great attention is being paid to quantitative urine work, as its importance is being recognised. I have worked with a view to helping in the process of simplifying quantitative methods, believing that with simple yet reliable methods will come the workers who are most wanted—the doctors in charge of cases. None of the methods given in the paper are long except three—those for the sulphates, uric acid and purin bodies; and for clinical work even these may shortly be abandoned for one of the centrifugal methods foreshadowed in this paper. I hope when the necessary tables for these processes have been completed that it will be possible to finish a complete quantitative urinary analysis in one hour, including the estimation of the total nitrogen, extracts, acidity, urea, uric acid, purin bodies not uric acid, preformed ammonia, phosphates, chlorides, sulphates both preformed and conjugated, and the urobilin.

I venture to think that an hour so spent and results so obtained will by the insight given into the inner working of that complex factor, the human body, repay the labour expended.

Estimation of Urinary Acidity.—The total acidity of urine may be estimated in many ways; but as the results obtained

by different procedures differ considerably, whichever method is adopted should be adhered to for all analyses.

The simplest method is to titrate a given quantity of urine, say 20 c.c., with a decinormal alkaline solution, till a drop of the titrated urine turns neutral litmus paper slightly blue. The number of c.c. of decinormal solution used, say x multiplied by $\frac{1000}{20}$, and by $\frac{1}{10}$, *i.e.*, by 5, will give the amount of urinary acidity per litre of urine in terms of normal alkaline solution.

A more satisfactory and still simple method is that introduced by Folin; it is based on the principle that phenolphthaleine, which while otherwise an unsatisfactory indicator in estimating urinary acidity, owing to its slow end reaction in the presence of ammonia salts in the latter, is rendered an eminently satisfactory one when these are removed by the addition of an oxalate of potassium.

The oxalate of potassium also precipitates any calcium salts present, which, by combining with the P_2O_5 to form basic phosphates and liberating free phosphoric acid, increase urinary acidity. To determine the total acidity, place 25 c.c. of urine in a flask (dilute if the urine is high coloured), add a few drops of phenolphthaleine, and from 15 to 20 grms. of oxalate of potassium, agitate the flask well and titrate *at once* with a decinormal alkaline solution till a faint rose colour is obtained. The number of c.c. of decinormal alkaline solution used, multiplied by 40 or by 4, will give the amount of urinary acidity in terms of decinormal and normal alkaline solutions respectively.

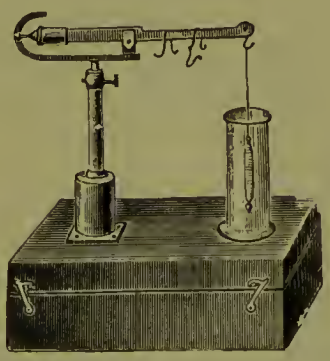
The Extract.—The urinary solids may be estimated by evaporating and drying a small quantity of the urine over a water-bath, and then weighing; but this is a long and laborious process, and a much simpler and perhaps equally accurate one is to take the specific gravity in a specific gravity bottle or Westphaal's balance (urinometers are hopelessly inaccurate), and multiply the last two figures by Häser's coefficient (2·33) to obtain the solids per litre.

The Westphaal balance (fig. 5) is based on the principle that a body immersed in a liquid loses a part of its weight equal to the weight of the displaced liquid.

The apparatus consists of a beam balanced on a stand. The free end of the beam has a hook from which a glass plummet is suspended; the other end of this beam ends in a point, which when the machine is regulated is exactly opposite a projecting point on the stand. The upper edge of the beam is divided by notches into a graduated scale, on which various-sized riders representing hundred, ten and unit weights respectively may be placed. The balance, with the ten weight placed on the hook at the end of the beam, is balanced exactly if distilled water is placed in the glass, the head of the plummet then remaining just immersed in the water.

If urine be placed in the glass instead of water the balance will be upset, and riders of various sizes will have to be placed on the notches of the beam to redress it. The size of the riders required and the notches in which they will have to be placed to restore the balance (*i.e.*, bring the two points shown in the picture opposite each other) will give the specific gravity of the urine.

The balance is standardised for a temperature of 15° C., and Purdy recommends that one figure should be added to the specific gravity found for every 7° of temperature above 15° C.—say specific gravity at 22° C. is 1019, ∴ true specific gravity is 1020. The last two figures, multiplied by 2·33 (20 × 2·33), equals 46·6, the amount of solids present per litre of urine.



N.B.—For this figure, as well as for figs. 6, 9 and 10, I am indebted to Messrs. Baird and Tatlock, of Cross Street, Hatton Garden, who very kindly lent me the woodcuts.

FIG. 5.—WESTPHAAL'S BALANCE.

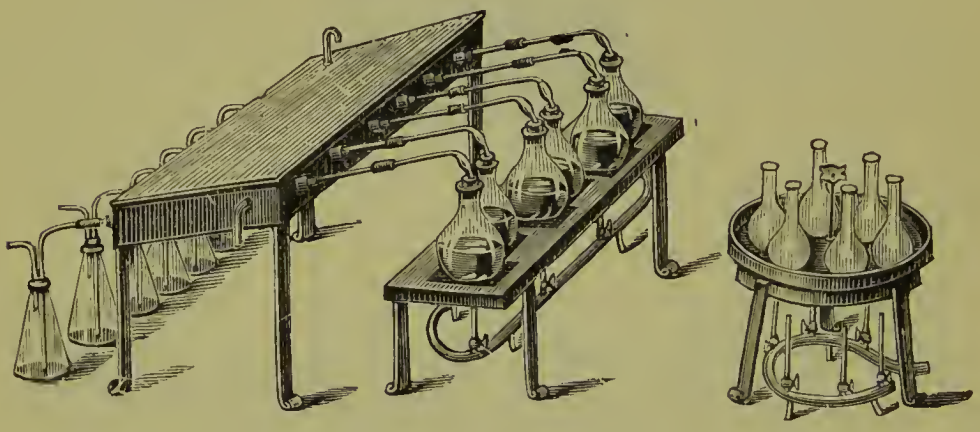
The Total Nitrogen.—The simplest and most practical method of estimating urinary nitrogen is by the well-known Kjeldahl's method, which is based on the principle that if strong sulphuric acid be added to a nitrogenous fluid and heated, ammonium sulphate, carbonic acid and water are formed. The ammonia can be recovered from the ammonium sulphate by distillation, and estimated. From the amount of the NH_3 present the N. can easily be calculated.

Procedure.—To 5 c.c. of urine in a long-necked flask add 10 c.c. of strong sulphuric acid and 5 c.c. of a 30 per cent. solution of neutral oxalate of potassium (which hastens the action of the sulphuric acid on nitrogenous matter). Place a balloon-shaped stopper in the flask mouth to prevent loss of the sulphuric acid fumes formed on heating the flask. Heat at first moderately and afterwards strongly, till the fluid in the flask is decolourised. The flasks used for boiling are seen on the right-hand side of the accompanying illustration.

Now cool the flask and add distilled water slowly, to 200 or 300 c.c., add a few drops of methyl orange or other indicator to the acid fluid

and then an alkaline solution such as a 20 per cent. caustic soda, till the indicator shows that the fluid is now decidedly alkaline. Attach the flask to a distilling apparatus (such as that seen in the picture), into the receiving flasks of which 50 c.c. of a decinormal solution of sulphuric acid have been placed. Now heat the flask containing the alkaline ammonia solution and allow the ammonia to distill over into the $\frac{N}{10}$ acid solution. Titrate the acid solution after all the ammonia has distilled over, against a $\frac{N}{10}$ alkaline solution. The moment when the ammonia distillation is complete may be determined by applying some litmus paper to the tube of the distillation apparatus where it enters the flask containing the sulphuric acid. Each c.c. of $\frac{N}{10}$ acidity lost by the distillate represents 0·0017 grm. of ammonia and 0·0014 grm. of nitrogen.

Example.—After receiving the ammonia distilled from 5 c.c. of urine, 50 c.c. of a $\frac{N}{10}$ acid solution were neutralised by 20 c.c. of a $\frac{N}{10}$ alkaline solution; ∴ 30 c.c. of $\frac{N}{10}$ acidity lost; each c.c. = 0·0014 grm. nitrogen; ∴ 30 c.c. = ·042 grm. nitrogen in 5 c.c., or $\frac{·042 \times 1000}{5}$ = 8·4 grms. nitrogen per litre of urine.



a. *b.*

FIG. 6.—KJELDAHL'S APPARATUS. *a*, Distillation apparatus; *b*, boiling flasks.

Urea.—The most rapid method of estimating urea has already been described in my first paper.* Another method which while not nearly so rapid, is perhaps even more accurate, is that recently devised by Folin. This process depends on the fact that urea when heated along with magnesium chloride breaks up into an ammonium salt;† the amount of the latter

* See page 12.

† N.B.—This break-up of the urea is not due to a chemical union with the magnesium chloride evidently, but to the high temperature at which magnesium chloride boils when dissolved in its water of crystallisation alone (viz., 160° C.), causing a complete break-up of the urea to ammonia.

can easily be estimated, and from it the original amount of urea in the sample calculated.

Procedure.—Three c.c. of urine are placed in a 200 c.c. flask, the rubber cork of which is pierced by a glass condensation tube six or eight inches long. Twenty grms. of magnesium chloride and 2 c.c. of pure hydrochloric acid are added. The flask is now heated gently until the magnesium chloride dissolves in its own water of crystallisation, and then more briskly for ten minutes. Loss of water from the already highly concentrated solution is prevented by the glass tube, which condenses the steam and causes the drops of water so formed to drop back into the boiling fluid. When the drops of water falling back into the flask do so with a hissing noise, the flame under the flask is reduced, and the fluid boiled gently for half an hour. At the end of this time all the urea has been converted to an ammonium salt. After the flask has cooled somewhat, the ammonia is estimated by adding 500 c.c. of distilled water and an excess of alkali (8 or 9 c.c. of a 20 per cent. solution of caustic soda), and distilling the ammonia over into a known quantity of decinormal acid solution, as in the Kjeldahl process just described. The next step is to estimate the amount of acidity lost in the decinormal acid solution through the presence of the ammonia by titrating this with a decinormal alkaline solution. Before doing so, however, the acid-ammonia solution should be boiled to drive off any carbonic acid brought over by the distillation.

As by this method the preformed ammonia is included in the result, it must be eliminated by (1) estimating the preformed ammonia by Shaffer's process and deducting its amount from the amount found here; or (2) precipitating the ammonia along with other urinary nitrogenous bodies by aid of a 10 per cent. solution of phosphotungstic acid. If very accurate results are required the latter method is probably the best, as undoubtedly small amounts of the creatinin and other extractives appear to be broken up to ammonia by the action of the magnesium chloride and high temperature. Each c.c. of decinormal acid solution neutralised by the ammonia distilled over represents 0·0017 grm. of NH_3, 0·0014 grm. of nitrogen, and 0·0060 grm. of urea.

Example.—The ammonia given off by the 3 c.c. of urine neutralises 9 c.c. of decinormal acid solution: ∴ 1 c.c. would neutralise 3 c.c. of decinormal acid solution: ∴ 1,000 c.c., or 1 litre, would neutralise 3,000 c.c. of decinormal acid solution. Now 1 c.c. of decinormal acid neutralised = 0·0017 grm. ammonia and 0·0060 grm. urea: ∴ the amount of ammonia and urea per litre of urine would be 0·0017 × 3,000. or 5·1 grms. ammonia, and 0·0060 × 3,000, or 18 grms. urea respectively.

Suppose the preformed ammonia has been estimated by the Shaffer process to be ·567 grm. per litre (which is equal to 2 grms. urea), ∴ the amount of urea alone in the 3 c.c. of urine will be 16 grms. per litre. If the urine has been previously acted upon by phosphotungstic acid, which precipitates the ammonia, this deduction will not be necessary.

Note.—A method recommended by Folin, but which I have not as yet tried, might be adopted to estimate both the urea and the performed ammonia from the one operation. This is rendered possible by the fact

that the preformed ammonia of the urine appears to distil over before the ammonia salt formed by the magnesium chloride and heat method just described, in fact, is completely distilled over in the first forty-five minutes. Moreover, the rate of distillation of the ammonia derived from the urea is constant. Now if the distillation be divided into two periods of forty-five minutes each (water being added before the second distillation to replace the same amount lost by the first distillation), it is evident that the amount of ammonia distilled over in the first distillation, minus the amount of the second, will give the preformed ammonia of the urine operated on, and this amount when subtracted from the whole ammonia distilled over will give the NH_3 of urea.

Uric Acid and the Urates.—The most rapid and practical volumetric method of estimating the total urates present (*i.e.*, the uric acid + the purin bodies) is that of Haycraft Deroide as modified by Denigès.

This method depends on the fact that if a mixture of magnesium and silver salts in ammoniacal solution be added to urine the urates present take up some of the silver-magnesium mixture, forming a double urate with these metals, which is precipitated. Denigès, instead of estimating the amount of silver, and hence urates, directly from this precipitate—a long and laborious process used in the method of Haycraft Deroide—estimates the amount of silver left in the filtrate after precipitation has taken place. As the strength of the silver in the silver magnesium mixture used is known, as well as the proportions in which the urate and silver combine, an estimate of the amount of silver left in the filtrate after precipitation will give the amount of silver taken up by the urates, and hence the quantity of the latter present. The reason why an ammoniacal solution of silver and magnesium is used is that the ammonium present prevents the urinary chlorides taking up the silver.

The amount of silver present in the urine filtrate (after removal of the precipitated urate of silver) is estimated by adding a quantity of a cyanide of potassium solution equivalent to the amount of silver originally added to the urine. When equal quantities of equivalent strengths of potassium cyanide and silver nitrate are brought together, a solution of a soluble double cyanide of silver and potassium is formed, in which there is no excess either of silver or of cyanide. Owing to the removal by the urates of some of the silver solution originally added to

the urine, a cyanide solution of equivalent strength to this original solution, if now added to the filtrate, must produce an excess of cyanide in the mixture. This cyanide excess is a measure of the silver taken up by the urates in the silver urate precipitate ; it can be estimated by adding a silver solution of known strength till the excess is neutralised. The saturation of the cyanide solution by the silver is easily estimated, as any excess of silver forms an insoluble precipitate of silver iodide, if a little potassium iodide be previously added to the cyanide filtrate mixture.

Procedure.—Two solutions are prepared. *Solution A* is prepared by adding together equal parts of (1) an ammonia-magnesium mixture, and (2) a decinormal solution of silver.

The magnesium mixture (1) is made by dissolving 150 grms. of chloride of ammonium and 100 grms. of chloride of magnesium in three-quarters of a litre of strong ammonia in a flask. The mixture is then corked and warmed to 25° or 30° C. (under a warm water tap) and the volume completed to the litre. The decinormal silver solution (2) is made by dissolving 17 grms. of silver nitrate in a litre of distilled water. The ammoniacal silver-magnesium solution thus formed keeps well even in white bottles. The strength of the contained silver is $\frac{N}{20}$.

Solution B is a decinormal solution of potassium cyanide and is prepared by dissolving 17 to 18 grms. of potassium cyanide in distilled water. This solution is then titrated against a decinormal solution of silver, and diluted till 10 c.c. exactly neutralises 10 c.c. of silver solution, neither salt being in excess. In addition to the above solution, a 20 per cent. solution of potassium iodide is made up as an indicator to show when saturation of the cyanide solution by the silver has taken place. The least excess of silver present in this case is indicated by the formation of an insoluble white precipitate of silver iodide.

To 100 c.c. of urine add 25 c.c. of solution A (the ammoniacal magnesium-silver solution), filter off the precipitate of urate of magnesium and silver formed. Take 100 c.c. of the filtrate representing 80 c.c. of urine ($\frac{100}{125}$ of 100 = 80), and 20 c.c. of the $\frac{N}{20}$ silver solution, and to this add 10 c.c. of solution B (potassium cyanide solution). Having added a few drops of the indicator (potassium iodide), the decinormal silver nitrate solution is dropped in from a burette, till a persistent cloudiness is obtained. Each c.c. of silver solution used represents 0·0168 grm. of urates expressed as uric acid ; or to make it simpler still, multiply the c.c. of silver solution used by ·21, and the answer will represent the quantity of urates present per litre of urine.

Example.—To the 100 c.c. of filtrate obtained from the mixture of 100 c.c. of urine, and 25 c.c. of ammonia-magnesium silver mixture, 10 c.c. of cyanide solution are added. The $\frac{N}{10}$ silver nitrate solution necessary to neutralise the excess of cyanide present is say 3·2 c.c.

Each c.c. of silver nitrate $\frac{N}{10}$ solution equals 0·0168 grm. of urate expressed as uric acid : ∴ 3·2 c.c. equal ·05376 grm. uric acid in 100 c.c. of filtrate (representing 80 c.c. of urine), or ·05376 × $\frac{1000}{80}$, or ·672 grm. in 1 litre (which is the same amount as multiplying 3·2 by ·21).

Uric Acid alone.—There are a great many methods employed for estimating the uric acid of urine, but those that are very accurate are very laborious, and others that are quicker are not very accurate. Of the longer methods that of Hopkins and its modifications are the best and simplest; but I have found the copper process of Denigès to be both accurate and rapid. It has been adopted as the standard method in France.

In this method, and in Blarez' modification of it, the uric acid is precipitated from the urine as urate of copper, and the copper in the precipitate estimated. The combining proportions of urate of copper being known, the amount of uric acid is readily estimated when the copper in the combination has been calculated.

The phosphates present in the urine are first eliminated by the addition of a 16 per cent. solution of sodium carbonate. A colourless alkaline copper solution (made by decolourising some Fehling's solution with a little alkaline bisulphite) is now added. The uric acid of the urine is taken up by the copper, and an insoluble urate of copper precipitates out. The precipitate is collected on a filter and thoroughly washed with hot water. Collecting the precipitate by filtering is a tedious process unless suction is applied to the filter. (If a water-pump be used the filtering is very rapid and satisfactory results are obtained.) The urate of copper combination has now to be broken up and the amount of copper in the precipitate estimated, These processes may be carried out in one of two ways.

Denigès breaks up the urate of copper by putting the precipitate into a capsule and adding $\frac{1}{2}$ to $1\frac{1}{2}$ c.c. of hydrochloric acid, and heating. Blarez adds 10 c.c. of a 50 per cent. solution of sulphuric acid and uses a flask. Denigès estimates the amount of copper present by first adding 10 c.c. of ammonia to form a bright blue combination (copper salts in the presence of NH_3 are blue), and then decolourising this blue fluid by a decinormal solution of potassium cyanide (compounds of alkaline cyanide with copper are colourless).

It is evident from this that the amount of cyanide solution used will indicate the amount of copper present in the precipitate, and hence the uric acid.

Blarez estimates the copper in the precipitate by adding a decinormal solution of permanganate of potassium to the copper

sulphate solution until the appearanee of a faint rose colour* which persists for one minute.

Solutions required for Denigès' Method.—(1) Sixteen per ccnt. solution of anhydrous carbonate of soda. (2) Fehling's solution, to which a solution of sodium bisulphite has been added, till the Fehling fluid is decolourised. (3) Hypobromite of soda solution, such as that used for the urea estimation. (4) A $\frac{N}{10}$ solution of cyanide of potassium similar to that prepared for the estimation of the total urates.

Procedure.—Place in a measured glass 120 c.c. of urine and 12 c.c. of solution (1); filter off the precipitate of phosphates formed. To 100 c.c. of filtrate, equal to 90 c.c. of urine, add 10 c.c. of thc decolourised Fehling's solution; filter, and receive the copper urate precipitate on a small flat filter paper (an exhaust pump will be found of great use in accelerating the filtration, which is otherwise long and tedious). Thoroughly wash precipitate with hot water and then wash it off into a porcelain dish; add 1 to $1\frac{1}{2}$ c.c. of pure hydrochloric acid and hypobromitc solution drop by drop till the copper solution is of a yellowish tint. The total volume of washings should not exceed 40 c.c. Boil, add 10 c.c. of ammonia, which colours the solution deep blue, and when the boiling is brisk drop in the $\frac{N}{10}$ cyanide of potassium solution till the blue colour has disappeared. The number of c.c. of cyanide solution used, minus 0·01, multiplied by 11, gives the amount of uric acid in each litre of urine.

Solutions used and Procedure in Blarez' Modification.—(1) Sixteen per cent. solution of anhydrous carbonate of soda. (2) Fehling's solution, decolourised by addition of an alkaline bisulphite. (3) $\frac{N}{10}$ solution of permanganate of potassium.

Procedure.—To 37 c.c. of urinc in a glass add 5 c.c. of solution 1; and to the mixture, after shaking, 7 c.c. of thc decolourised Fehling's solution. After five minutes, filter, receiving the copper urate precipitate on a small filter paper. Thoroughly wash the precipitate two or threc times. Place thc precipitate and filtcr paper in a flask along with 150 c.c. of watcr, shakc to free the filter papcr, add 10 c.c. of a 50 per cent. solution of sulphuric acid, shakc wcll, now add the permanganate solution till a rose-coloured tint pcrsists for half a minute to a minute.

The numbcr of $\frac{c.c.}{10}$th of permanganate solution employcd multiplied by 2 gives the numbcr of ccntigrammes of uric acid containcd in a litre of urinc.

The Method of Estimating the Urinary Chlorides is praetieally the same as that employed for the ehlorides in water (Mohr's method). It is not advisable, however, to aet direetly on urine, owing to (1) its eolour rendering the end reaction diffieult to perceive, and (2) the fact that there are other bodies

* N.B.—The permanganate solution gives off oxygen to the uric acid in the solution, and is thereby reduced and decolourised. The reappearance of the red colour of the permanganate indicates that the uric acid is oxygen saturated. The oxygen absorbing power of uric acid being known, the amount of the latter can be calculated.

beside the urinary chlorides which affect the titrating silver solution (viz., organic matters—extractives and albumens). The colour difficulty is easily got rid of by diluting the urine, while the organic matter may be destroyed by means of permanganate of potassium in the presence of an acid. An alkali (pure carbonate of lime) must be added to neutralise the urine again, as acidity vitiates the process. The principle on which Mohr's process is based is, of course, that if a chromate be added to the fluid to be analysed and a solution of silver dropped in, the latter is taken up by the chlorides present and only unites with the chromic acid to form a red chromate of silver when the chlorides are exhausted.

Procedure.—To 7·1 c.c. of urine, well diluted, add 2 c.c. of weak sulphuric acid ($\frac{N}{10}$ will do), boil gently, add permanganate of potassium till a yellow colour is present (the organic matter is now oxidised). Now add a pinch of carbonate of lime, which will not only neutralise the fluid, but will precipitate any oxalates which may have been formed by the action of the permanganate. Add a few drops of a chromate of potash solution and titrate with a decinormal solution of silver nitrate till a faint red colour is apparent. The number of $\frac{1}{10}$ c.c. of silver solution used divided by 2 gives the amount of chloride in grammes per litre of urine.

The Phosphates.—The total phosphoric acid in the urine is estimated by the nitrate of uranium method, the result being expressed in terms of P_2O_5 (anhydrous phosphoric acid). This process depends upon the fact that if a uranium salt such as the nitrate or acetate be added to urine, it combines with the phosphates present to form a phosphate of uranyl: the amount of uranium salt necessary to saturate the phosphates present is an index of the quantity of the phosphates present in the urine. Saturation of the phosphates is to be considered complete when such an indicator as cochineal or potassium ferrocyanide is attacked by the presence of free uranium nitrate.

The nitrate salt of uranium is not the best one to use for titrating, as free nitric acid is liberated during the titration of the urine. As this result can be completely checked by the addition of a little nitrate of sodium, however, it is better to use this salt than the more expensive acetate of uranium.

Procedure.—The following solutions are prepared:—

Solution A. Dissolve 40 grms. of nitrate of uranium in 600 or 700 c.c. of distilled water. As nitrate of uranium often contains free

nitric acid, this latter must be got rid of by the addition of a little ammonia (which on addition forms a precipitate) and some acetic acid (which dissolves the precipitate formed). As nitrate of uranium is never pure enough to enable a solution of required strength to be made from it directly, solution A has to be corrected by titrating it against a solution containing phosphoric acid in known strength. Such a solution may be obtained by dissolving 3·24 grms. of acid ammonium phosphate, or 5·887 grms. of soda ammonium phosphate, in a litre of water. Fifty c.c. of this solution, containing ·01 grm. of P_2O_5, are titrated along with some cochineale and acetate of soda against the uranium solution; the strength of the latter is now estimated (the most convenient strength of the uranium solution is for 1 c.c. of it to be equivalent to ·005 grm. of P_2O_5).

Solution B consists of 50 grms. each of acetate of sodium and of acetic acid dissolved in half a litre of water.

Solution C is the indicator used (tincture of cochineale).

To 50 c.c. of urine in a porcelain dish add 2 or 3 c.c. of the acetate of soda solution and a few drops of tincture of cochineale. Gently heat the dish (to about 80° C.) and pour in the uranium solution from a graduated burette till a bright green colour is apparent. As each c.c. of uranium solution equals ·005 grm. of P_2O_5, the amount of anhydrous phosphoric acid present in each litre of urine will be found by multiplying the number of c.c. by ·005 and 20; say 10 c.c. of uranium solution have been used for the 50 c.c. of urine, then $\frac{10 \times \cdot005 \times 1000}{50}$, or 1 grm., will be the amount of P_2O_5 present per litre of urine.

The Sulphates.—The only urinary sulphur estimations which are clinically practical and of value are those of the total acid sulphates and the conjugated sulphates. While the mineral sulphates may be precipitated by a salt of barium, and estimated as barium sulphate, the phenol sulphates have first to be dissociated by the action of heat and a strong mineral acid before they will yield up their SO_3 to a barium salt. This fact permits of the amount of mineral and organic or conjugated sulphates being estimated separately.

Procedure. — Estimation of Total Sulphates. — Boil 50 c.c. of filtered urine along with 5 c.c. of pure hydrochloric acid for fifteen minutes, add 10 c.c. of a hot 10 per cent. barium chloride solution, filter through a small filter paper of known ash; wash the filtrate thoroughly till the washings give no precipitate with a silver nitrate solution; dry and calcine the filter paper and precipitate, in a shallow previously weighed platinum dish. A drop or two of nitric acid aids calcination. Now cool the capsule and weigh carefully. The difference in weight between the capsule alone and the capsule + sulphate ash, multiplied by 0·34326, and again by 20 gives the amount of total sulphates in the litre of urine.

Estimation of Conjugated Sulphates.—To 125 c.c. of urine add an equal volume of a mixture of two volumes of barium hydrate and one of

barium chloride solution, both saturated. The precipitate, which consists of the mineral sulphates, is removed by filtration, and 200 c.c. of the filtrate, representing 100 c.c. of urine, boiled for fifteen minutes, along with 20 c.c. of pure hydrochloric acid. The precipitate of sulphates derived from the organic sulphates is collected, incinerated, and weighed, as in the case of the total sulphates, and similarly estimated. The difference between the total and the conjugated sulphates gives the amount of mineral sulphates present.

Carbon of Urine.—The carbon contained in a given sample of urine is estimated by breaking up the carbon compounds by means of sulphuric acid and collecting the carbonic acid gas which is evolved in a tube containing caustic potash. A carbon- and moisture-free air is then driven over the urine

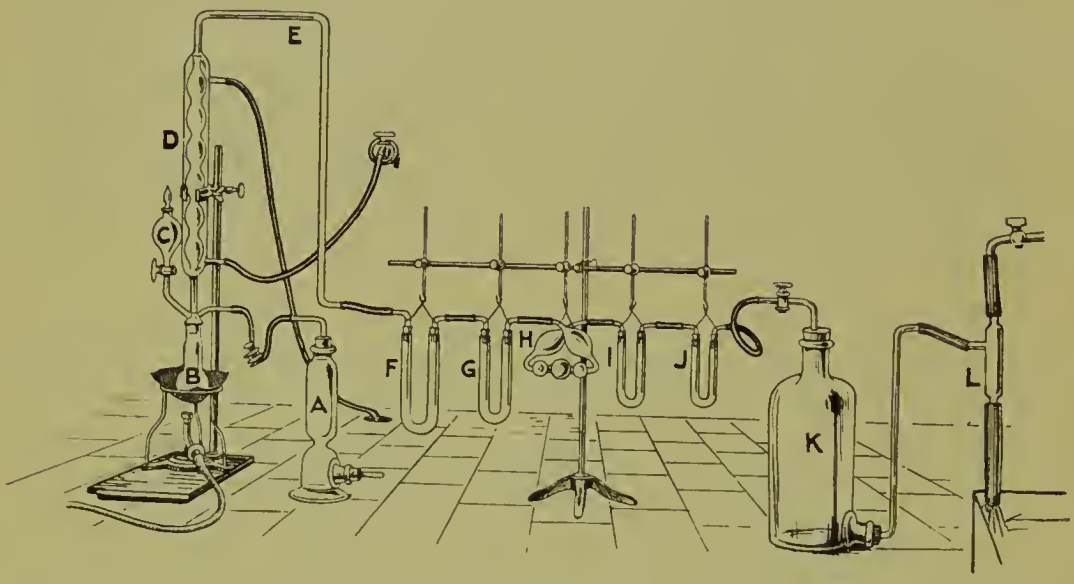


Fig. 7.

towards the potash tube to ensure the collection of all the carbonic acid. The method and apparatus used in that of Desgrez, and is as follows: 10 c.c. of urine and 10 grms. of chromic acid are placed in the 100 c.c. bulb marked B., to which are attached by means of a glass stopper three tubes; one tube leads to a stand (A) containing soda lime; a second tube leads up to a bulb (C), into which 25 c.c. of strong sulphuric acid are placed, the acid being allowed to drop slowly into the bulb containing the urine; the third tube leads up to a condenser (D), which is necessary to prevent the CO_2 gas

passing over too hot and too moist into the rectangular tube E. The first U-shaped tube (F) contains pieces of pumice stone saturated with strong sulphuric acid, in order to absorb any moisture coming over with the evolved CO_2. The second U-shaped tube (G) contains ferrocyanide of potassium and borate of soda, which absorb any chlorine or hydrochloric acid which may have been evolved from the urine. The spiral tube (H) is filled with caustic potash to absorb the CO_2 of the urine. Tube I contains pumice stone soaked with more caustic potash, and serves as a control to tube H; while the last tube (J) is filled like the first tube (F) with pumice stone saturated with sulphuric acid. This tube as well as bottle K are used to prevent any moisture getting access to the potash tubes (H and J) from the water-pump (L).

Procedure.—The potash tubes H and J are detached, weighed, and reattached. The 10 c.c. of urine and 10 grms. of chromic acid (which is necessary to retransform the sulphurous acid formed by the action of the H_2SO_4 on organic matter, into sulphuric acid) are acted on by the 25 c.c. of sulphuric acid gradually introduced from the flask C. The flask B is then gently heated. The heat applied should be short of that necessary to cause boiling till towards the end of the operation; by this means the CO_2 is slowly and steadily driven off from the urine (it should be possible to count the bubbles of gas as they come off). When all gas has been driven off a current of air is passed through the apparatus for twenty minutes; this air is deprived of both carbon dioxide and moisture by passing through the soda lime (bottle A). At the end of the operation, which lasts about two hours, the potash tubes (H and J) are again weighed. The difference between the weights of the potash tubes (1) before and (2) after the operation, indicates the amount of CO_2 absorbed by the caustic potash, and hence present in the urine. The amount of carbon is $\frac{3}{11}$ of this amount.

Ammonia.—Shaffer's vacuum method of estimating ammonia was illustrated and mentioned in my first paper on urinary analyses, but as the details of the process were not given they are inserted here.

This method consists in driving off the urinary ammonia by boiling urine in vacuo along with an alkali, which displaces and drives off the ammonia; the latter is collected in a decinormal solution of sulphuric acid, and the acidity lost by the acid determines the amount of ammonia present in the urine. As the urea of urine gives off ammonia if the temperature exceeds 60° C., the boiling point has to be kept below that temperature: this is effected by means of the vacuum method and methyl

alcohol. The addition of sodium chloride to the urine also helps to prevent any urea decomposition.

Procedure.—Place in the small flasks (seen suspended in fig. 8) 100 c.c. of a $\frac{N}{10}$ solution of sulphuric acid. In the large flask shown in the water-bath place 50 c.c. of urine, 50 c.c. of methyl alcohol and

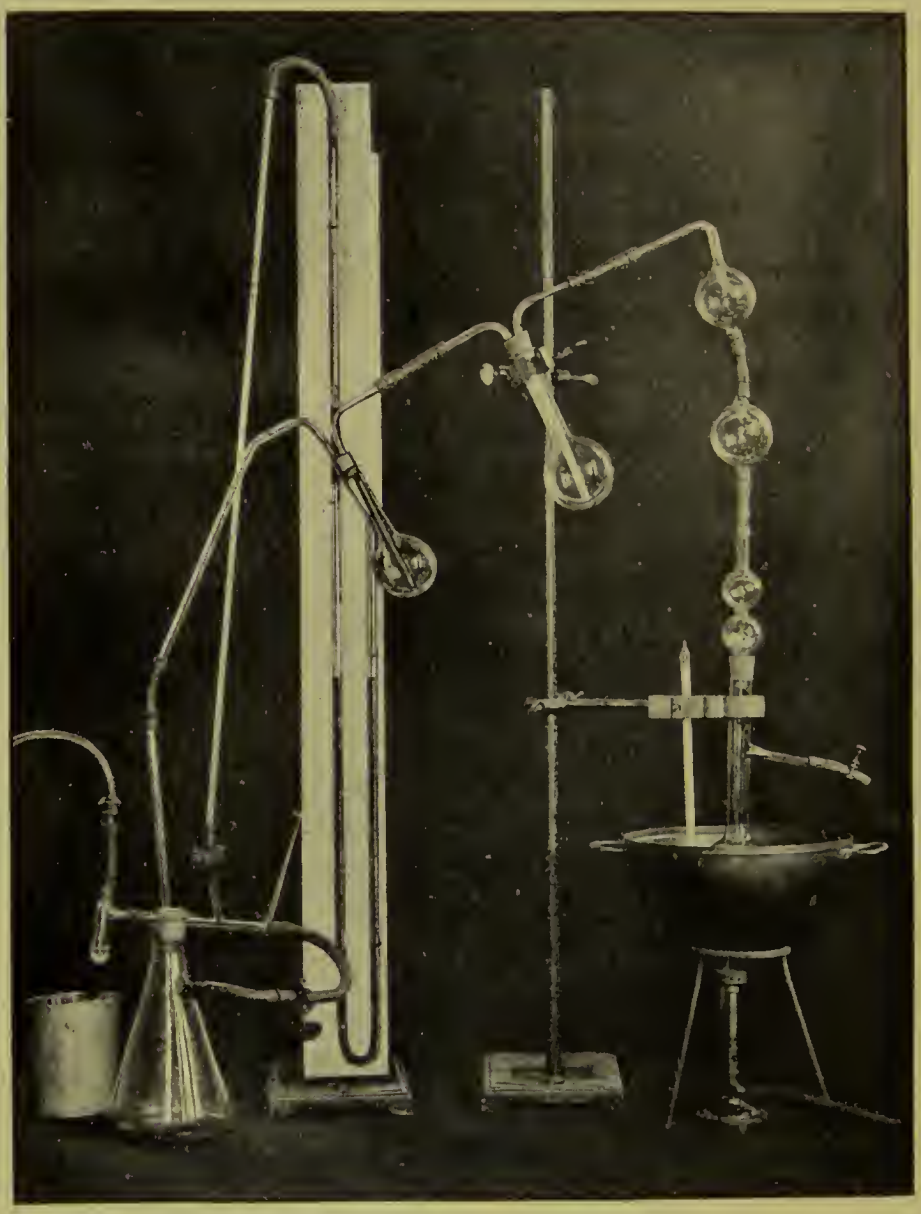


Fig 8.—Shaffer's Ammonia Apparatus.

20 grms. of sodium chloride; place 3 or 4 grms. of sodium carbonate in the flask and attach this rapidly to the two small glass bulbs seen just above it in the photo. The apparatus used and figured in the illus-

tration is connected to a water pump and to a mercury gauge. When the urine and other ingredients have been placed in the large flask this is placed in a water-bath, and the rubber tube seen on the right of the photo connecting the flask with the air pinched up. The air in the apparatus is now exhausted by turning on the water pump seen on the left of the photo. When the mercury gauge registers 20 m.m. of mercury the water-bath is heated. Boiling commences usually at or below 40° C. and the temperature must not be allowed to exceed 45° C. The four small glass bulbs seen in the photo above the boiling flask are simply intended to prevent any of the alkali contained in the boiling flask from being drawn into the decinormal acid solutions in the small glass flasks and so spoiling the result. After boiling for twenty minutes a current of air is allowed to pass through the apparatus, the acid-containing flasks are detached, and their acidity determined by titration with a $\frac{N}{10}$ alkaline solution. Any loss of acidity caused by the ammonia distilled over is determined; each c.c. of acidity lost represents 0·0017 grm. of NH_3. (Use alazarine red or methyl orange as indicators.)

Example.—The $\frac{N}{10}$ acid of the small flasks when titrated after the process only equals 50 c.c. of $\frac{N}{10}$ alkaline solution; therefore 100 − 50, or 50 c.c., of $\frac{N}{10}$ acidity have been neutralised by the ammonia driven off from the urine. Each c.c. of acidity lost, however, equals 0·0017 grm. NH_3; therefore, 50 c.c. equals 0·085 grm. NH_3 found in 50 c.c. of urine, ∴ the amount per litre must be $\frac{0{\cdot}085 \times 1000}{50}$, or 1·7 grms.

Sugar.—Of the many volumetric methods of estimating the sugar in urine the best are those of Gerrard and Purdy's modification of Pavy—both modifications of the well-known Fehling process. In Fehling's original method—still largely used—the end point of the process is unsatisfactory, as it is difficult to estimate when the blue copper solution has been completely decolourised, owing to the constant formation of the red oxide of copper. Gerrard obviates this difficulty by adding a cyanide to the copper solution, which forms a colourless compound with the copper oxide reduced from the sulphate, thus giving a clear reaction. Pavy's modification is to produce a similar colourless end reaction with the aid of ammonia (which dissolves the copper oxide precipitate). Purdy has altered the composition of the Pavy-Fehling solution, substituting glycerol for the sodic tartrate employed.

Gerrard's Process.—Solutions required:—

(1) *Fehling's Solution.*—A mixture of equal parts of two solutions: (*a*) 69·25 grms. of pure copper sulphate, powdered and dried; 1 c.c. of pure sulphuric acid, and water to the litre; (*b*) 350 grms. of Rochelle salt are dissolved in 700 c.c. of water, 100 grms. of caustic potash are added, and water to the litre.

(2) A 5 per cent. solution of potassium cyanide.

Procedure.—Place 10 c.c. of freshly prepared Fehling's solution in a porcelain dish and add 40 c.c. of water, heat to boiling, add the cyanide solution carefully till the blue colour of the Fehling is all but gone (excess of cyanide must be avoided). Now add 10 c.c. of Fehling to the faintly blue mixture, and the solution is ready (a stock of this may be made up). These 10 c.c. of Fehling are decolourised by ·05 grm. of glucose. Dilute the urine to be analysed twenty times and drop it into the boiling copper solution from a burette till the blue colour has completely disappeared ; the boiling must be brisk during the whole process.

Say 20 c.c. of urine diluted twenty times decolourises 10 c.c. of Fehling's solution equal to ·05 grm. of sugar, ∴ the percentage of sugar in the urine is $\frac{20 \times 100}{20} \times \cdot 05$, or 5 per cent.

Pavy's Process modified by Purdy.—The procedure here is similar to the above, but the copper solution used is, pure copper sulphate 4·752 grms., potassium hydroxide 23·50 grms., glycerol 38 c.c., strong ammonia 350 c.c., water to the litre. 35 c.c. of this solution are equal to 0·02 grm. of glucose.

The glycerol is substituted for the unstable Rochelle salt used in Fehling's and Pavy's solutions.

Thirty-five c.c. of the above solution are boiled in a flask, and the diluted urine dropped in from a burette. As it takes 0·02 grm. of glucose to decolourise the 35 c.c., the amount of urinary sugar can readily be ascertained.

Example.—Say 20 c.c. of urine diluted ten times (*i.e.*, 2 c.c. urine) decolourises the 35 c.c. of copper solution, ∴ there will be 0·02 grm. glucose in 2 c.c., or 10 grm. in 100 c.c. = 10 per cent. sugar.

B. Oxybutyric Acid.—The most simple and rapid method of estimating this acid is by the polariscope.

B. oxybutyric acid is lævogyric, so by noting the amount of left deviation—when this is present—the amount of the acid can be ascertained. As this acid occurs chiefly in urine containing sugar, the influence of the latter substance, which is dextro-rotatory, must be allowed for. A 100 per cent. solution of B. oxybutyric acid deviates light 24° to the left, while glucose in similar strength deviates light 58·3° to the right (sodium flame). Each degree of a Laurent polariscope with sodium flame is graduated to equal 2·27 grms. of sugar, and 4·64 grms. of B. oxybutyric acid per litre of urine. With a Schmidt and Hensch's polariscope the amounts of sugar and acid per degree of the instrument are 3·34 and 6·9 grms. respectively. A sodium flame is used with Laurent's polariscope, and white light with the Schmidt and Hensch.

Procedure.—To estimate the oxybutyric acid in a diabetic urine, in say a Laurent's polariscope, the following method may be employed. To

100 c.c. of urine add 10 c.c. of a saturated solution of subacetate of lead; this removes the urinary pigments and clarifies the urine, enabling one to see through a long tube full of it. The 20 c.m. tube of the polariscope is now filled completely with the clarified urine, the tube placed in the polariscope, and the light looked at through the tube and prisms of the instrument. The amount of deviation caused by the sugar and acid together is noted. The amount of rotatory action on light rays caused by a substance is indicated by the nature of the shadow thrown on a finely bisected disc near the eye-piece of the instrument.

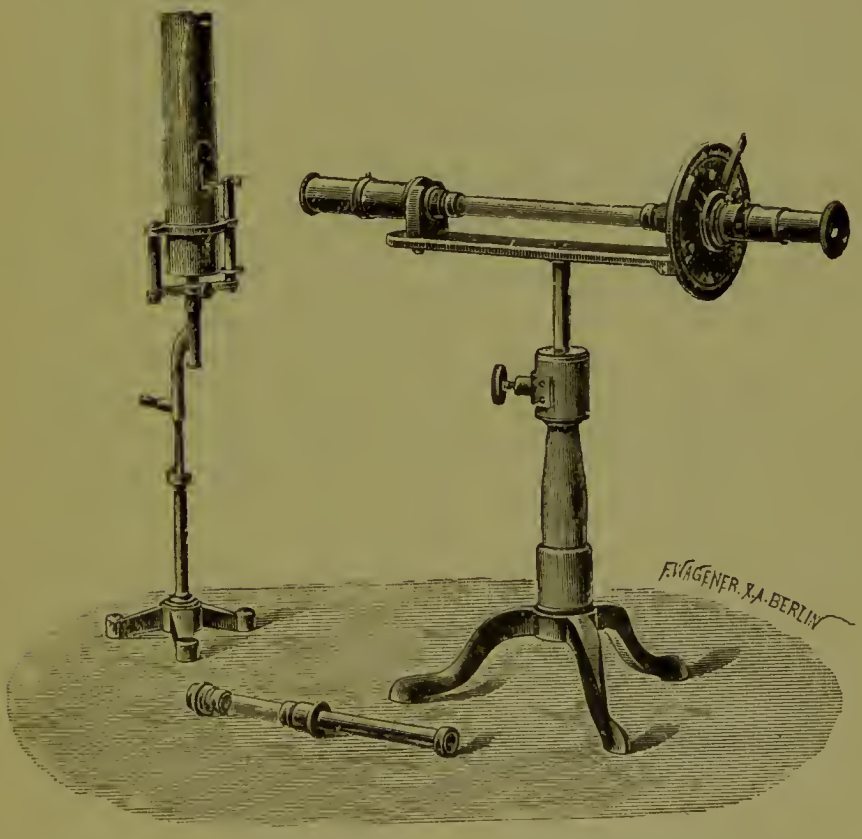


FIG. 9.—A cheap form of Polariscope suitable for estimating the sugar in urine. The 20 c.m. tube is in position in the instrument. The light used here is a sodium flame.

If a tube of distilled water (which has no polarising action on light) be placed in the polariscope, the shadows on the two sides of the bisected disc are equal; if a dextro-rotatory substance in solution, such as sugar, now takes the place of the water in the tube, a dark shadow varying in intensity with the amount of sugar present is seen on the right half of the bisected disc. With a levo-rotatory substance like B. oxybutyric acid a shadow is thrown on the left half. The amount of rotation present and hence the amount of the substance causing it is estimated by noting the number of degrees through which a quartz disc has to be moved to neutralise the polarisation, and hence restore the shades on the halves of the disc.

The amount of rotation necessary to obliterate the shadow on the right disc, caused by the diabetic urine, being noted, the amount of sugar in the urine is estimated by the cupric method. The amount of deviation caused by definite amounts of sugar being known, it will be evident that if sugar alone is present in the urine, this should be present in amount sufficient to account for the deviation of light shown by the polariscope. If the amount of polaric deviation is less than it ought to be, from the amount of sugar in it, the difference is due to the influence of B. oxybutyric acid, which is strongly lævogyric. The difference multiplied by 4·64 gives the amount of B. oxybutyric acid in grammes per litre present in the urine. (Laurent's polariscope.)

Example.—Suppose the 20 c.m. tube full of urine throws a shadow on the right half of the disc, which it requires 15° of rotation to obliterate. Suppose, further, the sugar in the urine has been estimated to be 55·4 grms. per litre. As we know that each 2·27 grms. of sugar per litre of urine in a 20 c.m. tube diluted $\frac{1}{10}$ equals 1° in a Laurent instrument, ∴ there should be 20° of dextro-rotation and not 15°; 20° — 15°, or 5° represents the influence of the left rotation of the B. oxybutyric acid. It takes 4·64 grms. per litre of this acid to cause 1° of left rotation in a Laurent polariscope, therefore the amount of B. oxybutyric acid present must be 5° × 4·64, or 23·2 grms. per litre.

More accurate but longer methods of estimating B. oxybutyric acid are: (1) Fermenting the urine with yeast overnight and thus getting rid of any sugar present: the polaric deviation present will be due to oxybutyric acid alone, and its amount will indicate the quantity of B. oxybutyric acid present. (2) If urine is mixed with strong sulphuric acid and the mixture strongly heated and distilled, any oxybutyric acid present is converted to crotonic acid, which is distilled over. Crotonic acid is oxybutyric acid—one molecule of H_2O, and by estimating the crotonic acid formed the amount of B. oxybutyric acid can be determined.

Albumen.—The method which has held the field up to the present for the quantitative estimation of albumen has been the ponderal one. This consists in precipitating urinary albumen by trichloracetic acid, filtering, washing the precipitate of albumen, drying and weighing. While undoubtedly an accurate method, it has no great advantages in this respect over the centrifugal method of Purdy, to be described later on, and is incomparably longer and more difficult. Latterly I have used the centrifugal method alone.

Qualitative Estimation.—Brief details of the methods employed for the qualitative examination of the urine may be of interest.

The Albumen has been estimated usually by the nitric acid test, as it yields other information besides the question of albumen. The best method to employ is to place a little urine in a conical urine glass and add the nitric acid by a pipette, the point of which is placed near the bottom of the vessel before

the nitric acid is allowed to flow out; the heavier acid floats the lighter urine up, and a very distinct line of junction is seen. A white haze to band at this junction, signifies albumen; a white ring half inch or more higher up in the urine shows excess of uric acid; a play of colours where the acid joins the urine denotes the presence of bile pigments, and a dark brown band in the same place an excess of urobilin. Boureau's reagent, a mixture of 5 parts of sulphosalicylic and 15 parts of sulphophenic acid in 100 parts of water is a useful and sensitive reagent. The most sensitive test for albumen in the urine is that by Tauret's reagent, which consists of a mixture of 4·06 grms. of perchloride of mercury, 9·66 grms. of potassium iodide, 60 c.c. of crystallised acetic acid, with distilled water to 192 c.c. The reagent gives a precipitate with the albumoses as well as albumen.

Albumoses, if present alone in urine, may be detected by the formation of a ppt. with Tanret's solution. If albumen is also present this must be precipitated by heat, and the ppt. removed by filtering the urine while hot.

The test I have usually adopted for *indican* in the urine is that of Loubiou. To 2 or 3 c.c. of urine add an equal quantity chloroform, 1 c.c. or so of peroxide of hydrogen, and 3 or 4 c.c. of hydrochloric acid; mix and heat gently. If indican is present a blue colour appears in the chloroform layer of the mixture. The intensity of the colour present shows approximately the amount of indican.

Another similar and excellent method is to substitute sulphuric acid and persulphate of sodium for the hydrochloric acid and peroxide of hydrogen; a larger quantity of urine, 20 c.c., should be taken with 5 c.c. each of chloroform and the persulphate solution, and a few drops of the acid. No heat is required. (Amman's method.)

Bile Pigments have been estimated by the Gmelin-Rosenbach test. It consists in placing a drop of nitric acid on the damp portion of the filter paper through which urine has filtered. Surrounding the spot where the acid has been placed several faintly-coloured rings will appear—yellowish-red, violet, blue and green, in that order, from within outwards.

The most sensitive method of estimating bile pigments is that of Jolles, who has gone carefully into this subject of bile pigment testing.

In a 60 cc. burette place 50 c.c. of urine, a few drops of diluted hydrochloric acid, an excess of barium chloride, and 5 c.c. of pure chloroform. Shake the above mixture well, and leave for ten minutes. Remove the chloroform and precipitate by opening stop-cock (the chloroform and precipitate are at the bottom of the burette); the small amount of urine usually unavoidably removed at the same time does not affect results. Place chloroform and precipitate in a warm chamber or water-bath for five or ten minutes to evaporate the chloroform. Now add a few drops of nitric acid to the residue—the coloured rings above described are at once seen. This test reveals the presence of bile pigments when in 0·1 per cent. strength, while the Gmelin method unmodified does not give clear results unless 5 per cent. of bile pigments are present in the urine.

Bile Acids.—The simplest qualitative test of these acids is that of Hay. Owing to the increased superficial tension given to a fluid by the presence of bile acids, flower of sulphur, which when thrown on a dish containing normal urine, floats, will sink rapidly if bile acids are present. Another test is that of Oliver, based on the fact that bile acids precipitate peptones in acid solution.

If to 20 minims of urine 60 minims or 120 drops of a mixture containing ʒss. of peptone, grains 4 of salicylic acid, ʒss. of acetic acid, and water to ʒviii. be added, a slight and temporary opalescence is produced if there is no excess of bile acids present in the urine.

This method may be used for approximate quantitative work. Appended is Olliver's table.

Urine. Minims or drops of solution added to 20 mins. of urine produce opalescence.			Percentage of increase of bile salts over the normal.
Mins.		Drops.	Per cent.
45	..	90	50
40	..	80	66
35	..	70	83
30	..	60	100
25	..	50	240
20	..	40	300
15	..	30	400
10	..	20	600
5	..	10	1,200

Centrifugal Methods of quantitative estimation. It is convenient to group all centrifugal estimations for the urinary chlorides, phosphates, sulphates, albumen and other constituents of normal or abnormal urine under this heading. To Purdy, of Philadelphia, is due the credit of having first introduced the rapid and satisfactory method of centrifugalisation into urinary work.

Instead of the long volumetric method, or the still longer and more laborious gravimetric method, Purdy essayed to measure the precipitates of chlorides, phosphates and albumen,

formed by the addition of suitable reagents to the urine, and centrifugalised to a compact homogeneous layer in a graduated centrifugal tube.

Purdy's methods are as follows: A centrifugal machine, driven by electricity and capable of a speed of from 1,500 to 10,000 revolutions per minute is employed. The arms of the machine carry tube holders, in which are contained tubes of 15 c.c. capacity, graduated to tenths of a c.c. for the first 10 of the 15 c.c.—the first 5 c.c. being finely drawn out and further graduated in fortieths of a c.c. The radius of the arms and tubes (*i.e.*, distance from the central pivot to the ends of the tubes when held out horizontally) is $6\frac{3}{4}$ inches.

To estimate the urinary chlorides, phosphates, sulphates, and albumen (if the latter is present), four of the 15 c.c. centrifugal tubes are filled to the 10 c.c. mark with urine. To No. 1 tube is further added 1 c.c. of strong nitric acid and 4 c.c. of an $8\frac{1}{2}$ per cent. solution of silver nitrate (chlorides). To No. 2, 2 c.c. of a 50 per cent. solution of acetic acid and 3 c.c. of a 5 per cent. uranium nitrate solution (phosphates). To tube 3, 5 c.c. of a mixture containing barium chloride 4 parts, strong HCl. 1 part, and distilled water 16 parts (sulphates). To tube 4, 3 c.c. of a 10 per cent. solution of potassium ferrocyanide and 2 c.c. of a 50 per cent. solution of acetic acid (albumen). The above solutions are allowed to stand for three minutes, till the respective precipitants have formed, are then centrifugalised for three minutes, and the amount of the sediment formed by the various precipitates calculated in bulk percentage to the 10 c.c. of urine employed in each case. Each tenth of a c.c. of sediment thus becomes 1 per cent. bulk percentage; each $\frac{1}{40}$ c.c. (the first 5 c.c. are divided into fortieths) equals ·25 per cent. (bulk percentage).

Tables are given by Purdy which show that with the mineral constituents (chlorides, phosphates and sulphates) revolved at 1,200 revolutions of the centrifuge per minute, each $\frac{1}{40}$ c.c. (·25 bulk percentage) of chloride precipitate equals 0·03 per cent. of sodium chloride and 0·02 per cent. of chlorine. The same amount of sulphate precipitate equals 0·06 per cent. of SO_3, while each $\frac{1}{20}$ c.c. (*i.e.*, ·5 of bulk percentage of the phosphate precipitate) equals 0·005 of anhydrous phosphoric acid (P_2O_5), except the first two $\frac{1}{20}$ c.c., which each represent 0·02 per cent. of phosphates. With the tube containing the albumen precipitate revolved at 1,500 revolutions per minute, each $\frac{1}{40}$ c.c. (*i.e.*, ·25 bulk per centage) equals 0·005 per cent. of dry albumen.

The one disadvantage of Purdy's centrifugal method is his insistence on a mechanical (preferably electrical) centrifuge. If this condition were as essential as Purdy considers it, quantitative urinary analysis would be much restricted, as electrical centrifuges are expensive and somewhat difficult to work and manage where electricity is not laid on to a building. I have, however, by experiment and calculation found a means of working out quantitative analyses on the ordinary hand centri-

fuge used largely in laboratories in England, allowing at the same time advantage to be taken of the tables Purdy has drawn up, thus permitting anyone possessing the small "high-speed medical hand centrifuge" to work out quantitative urinary analyses very simply and rapidly, and with an accuracy sufficient for all clinical purposes.

The formula for centrifugal force is C. $= \frac{\text{v.}^2 \times \text{w.}}{\text{r.} \times 32{\cdot}2}$, v. standing for the velocity (feet per minute covered by the extremity of the centrifugal tube); w. for weight; r. for radius of arms of centrifuge, *i.e.*, length from pivot to tips of extended tubes. The 32·2 stands for gravity. The question to be solved, however, is not the centrifugal pressure at the apex of the tube, but the driving of the particles of the precipitate through the fluid and the conversion of the power of one variety of centrifuge to perform this into terms of another. The problem, therefore, becomes much simplified, weight and gravity can be eliminated from the equation, and the answer obtained by dividing the radius of the Purdy centrifuge by that of the instrument which one is using, and multiplying the result by the revolutions used to obtain his results. Thus Purdy obtained his results and formulated his tables of the chlorides, phosphates and sulphates with a centrifuge with $6\frac{3}{4}$ in. radius, and used a speed of 1,200 revolutions per minute: all that is now necessary is to divide this number ($6\frac{3}{4}$) by the radius of our own machine and multiply the 1,200 by the result.

The standard hand machine used in this country has generally an arm radius of 5 to 6 inches, and a spin of 20 to 60 revolutions to each turn of the handle. The machine I worked with had an arm radius of $5\frac{1}{5}$ inches, and a spin of 20 to each turn of the handle. Here $\frac{6\frac{3}{4}}{5\frac{1}{5}} \times 1{,}200$ (the revolutions Purdy employed in his chloride, phosphate and sulphate estimations), or 1,560 revolutions, were necessary in order to obtain similar centifugal force to that which he uses. Purdy employs his centrifugal force for three minutes to obtain his results, so by turning the handle of my machine $\frac{1560}{20}$, or 78 times a minute, for three minutes, I was enabled to make use of his tables. If a watch be placed on the table and 13 revolutions be made in every ten seconds, it will be found quite easy to keep up a regular speed and get constant results. This regulation of speed is not so easy if one tries to count the 78 revolutions in the whole period of sixty seconds.

A similar calculation to the above in the albumen estimation tables (when a speed of 1,500 on the Purdy machine has been used) would necessitate a speed of 1,970 revolutions, or 16 turns of the handle in ten seconds. As the tubes supplied with the hand centrifuge are generally 10 cc. tubes (graduated to $\frac{1}{10}$ of a c.c., except the two first c.c., which are graduated to twentieths), two-thirds of both the urine and the ingredients used by Purdy must be taken and the results obtained multi-

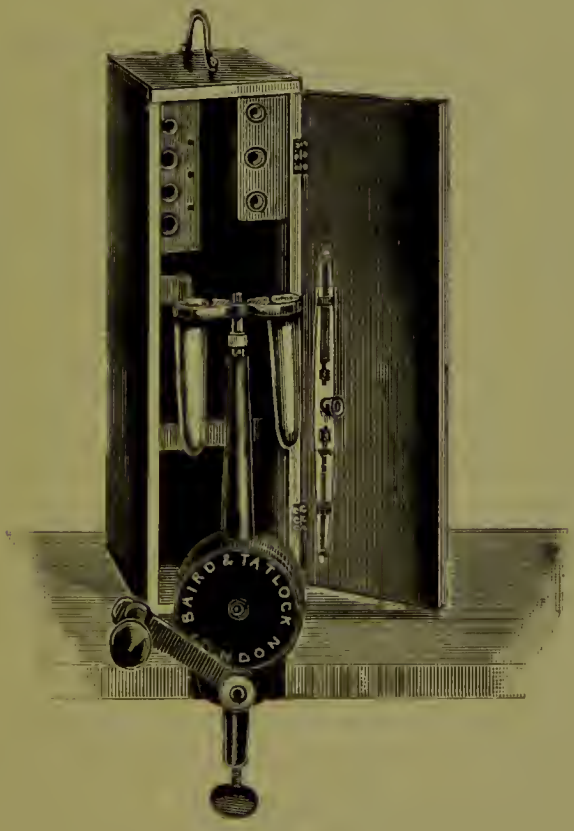


Fig. 10.—The high-speed hand centrifuge in general use in laboratories.

plied by $\frac{3}{2}$. It is better, however, to obtain the well-graduated 15 c.c. tubes used by Purdy.

I have endeavoured to further extend the use of the centrifugal method by working out methods for estimating the urinary uric acid, total purins, purins apart from uric acid and the conjugated sulphates. The results so far obtained have been very encouraging, but the amount of labour required to work out the necessary tables and check the results is considerable, and I have been unfortunately obliged to abandon

the work—I hope temporarily—before its completion. I may say that the method devised for estimating the total purins is based on the Haycraft-Denigès' procedure described earlier in the paper. Instead, however, of combining the two parts 1 and 2 of solution A, described on page 45, I first add the ammonia-magnesia mixture to precipitate out the phosphates, and then add the $\frac{N}{10}$ silver solution. The resulting precipitate of silver and purins is centrifugalised, and its amount calculated and checked by control experiments with the Haycraft-Denigès' method.

The uric acid (alone) is calculated on a method allied to the Denigès' procedure for calculating this body : the phosphates are eliminated by solution 1 containing carbonate of soda (page 47), and uric acid precipitated out from the filtrate by the addition of a given quantity of Fehling's solution acted on by an alkaline bisulphate. Uric acid is alone precipitated out under these conditions, and can be centrifugalised out as a urate of copper.

The total sulphates are estimated by treating the urine with strong hydrochloric acid in the presence of barium chloride and boiling for ten minutes, the resulting precipitate representing the total sulphates being centrifugalised and measured. Purdy does not use heat when estimating the total sulphates by the centrifugal method, and cannot, as far as I am aware, completely precipitate out his conjugated sulphates.

The conjugated sulphates are estimated in a similar manner to the total sulphates in the filtrate of a urine to which the chloride of barium and a little acetic acid have been added to precipitate out the mineral sulphates.

The above methods of estimating the total and conjugated sulphates appear—so far as I have gone—to give results sufficiently accurate for relative determination of the salts. The method is certainly a rapid and simple one.

Conclusions.—If accurate centrifugal methods can be devised, as now seems probable, for estimating most of the urinary constituents, quantitative urinary analysis will become so simple a matter as to be undertaken in all hospitals of any size where a hand centrifuge exists and a few chemicals are procurable.

While quantitative urine work is confined to a few metabolism experiments in selected laboratories we shall continue

to lose the great opportunities which we all have of finding out something of the conditions of metabolic exchange and body nutrition in various diseases; of organic resistance to disease, and the effect of diseases on the nutritional rhythm. The microbe has had considerably more attention paid to it than has the chemical nature and cell life of its occasional medium of growth—the human body.

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